STUDIES OF *ALTERNARIA* SPP. PATHOGENIC ON *CRUCIFERAE*

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INTRODUCTION

The nomenclature of the pathogens causing Alternaria leaf-spots of Cruciferae has been found to be very confusing in the literature. Several binomial and trinomial names have been applied; however, three pathogens, Alternaria brassicicola (Schw.) Wilt, A. brassicae (Berk.) Sacc., and A. raphani Groves and Skolko were considered and adopted as the causal organisms by Wiltshire (86) and accepted by Walker (77) and utilized by the writer.

The diseases, especially the ones caused by the first two pathogens, are world-wide in distribution. In the United States, cruciferous crops were grown on nearly two-hundred thousand acres at a cost of about 82,485,000 dollars in 1959. Of this, 30,670 acres at a value of 8,726,000 dollars were grown in Florida. The acreage, production, and value of cruciferous crops in the United States and Florida are given in Table 1. Alternaria leaf-spots are very common on crops in crucifer-growing areas. Although they are not destructive in the northern parts of the United States, they frequently do much damage there. They are especially destructive in the winter-grown southern crops in the fields or during transit. In Florida, the Alternaria leaf-spot diseases, along with downy mildew, have caused greater losses than all other diseases of cabbage.

This research was conducted to obtain more information regarding symptoms, the characteristics of the pathogens in artificial culture and pathogenicity as found on some cruciferous hosts in Florida.

TABLE 1

ACREAGE, PRODUCTION AND VALUE OF CRUCIFEROUS VEGETABLES GROWN FOR FRESH MARKET AND PROCESSING IN THE UNITED STATES AND FLORIDA IN 1958 AND 1959

Crop	Year	Acreage Acres		Production 1,000 cwt.		Value 1,000 Dollars	
		United States	Fla.	United States	Fla.	United States	Fla.
Broccoli	1958	39,930	*	2,192	*	16,953	*
	1959	40,750	*	2,260	*	17,437	*
Brussels Sprouts	1958	5,050	*	570	*	5,026	*
	1959	5,710	*	590	*	5,364	*
Cabbage	1958	119,460	*	21,166	*	39,685	*
	1959	119,060	18,000	19,043	2,700	44,734	5,566
Chinese Cabbage	1958	*	*	*	*	*	*
	1959	*	370	*	122	*	354
Cauli-flower	1958	30,250	*	4,646	*	16,622	*
	1959	27,200	500	4,120	50	14,250	220
Kale	1958	2,500	*	162	*	940	*
	1959	2,500	*	175	*	700	*
Radish	1958	*	*	*	*	*	*
	1959	*	11,800	*	519	*	2,586
Total	1958	197,190	*	28,736	*	79,226	*
	1959	195,220	30,670	26,188	3,391	82,485	8,726

*Data not available.

SOURCE OF MATERIALS

Plant Materials

Thirteen commercial varieties of the common vegetable crop plants of the Cruciferae family were purchased as seeds from local seed stores as listed in Table 2. The plants were grown in two outdoor plots about one-half mile apart on the campus of the University of Florida. They were also grown in six- and eight-inch clay pots filled with fumigated soil in a greenhouse. Seeds of varieties were arranged alphabetically according to their common names and were sown either directly in the plots in a series of consecutive rows or were sown in wooden flats and the seedlings were transplanted later. The percentages of seed germination varied from time to time, probably depending on their age. Generally, no infection which indicated seed transmission of any disease was observed on the seedlings. Plants were maintained in a thrifty condition by occasional applications of a commercial fertilizer. Aphids and cabbage loopers were controlled by spraying with Trlodan or Malothion, and downy mildew was controlled with Karathane sprays.

Pathogens

The pathogens were readily isolated from leaf lesions of various crucifers collected from different locations. Most collections were made near Gainesville; others were made from Bradenton and Hastings, Florida. The pathogens were also received as pure cultures from North Carolina, Arkansas, American Type Culture Collection, Ottawa, Canada, and other

TABLE 2

CRUCIFEROUS PLANTS USED IN THE EXPERIMENTAL WORK

Name		Source of Plants
Common and Commercial	Scientific	
Broccoli (BO) Green Sprouting	B.oleracea v.botrytis L.	Northrup, King Seed Company
Brussels Sprouts (BU) Improved Dwarf	B.oleracea v. gemmifera Zenk.	Northrup, King Seed Company
Cabbage (CB) Early Jersey Wakefield	B.oleracea v.capitata L.	Northrup, King Seed Company
Chinese Cabbage (CH) Chihili	B.pekinensis(Lour.)Rupr. or B.pe-tsai Bailey	Northrup, King Seed Company
Collards (CO) Georgia	B.oleracea v.acephala DC.	Northrup, King Seed Company
Cauliflower (CU) Early Snowball	B.oleracea v.botrytis L.	Northrup, King Seed Company
Kale (KA) Dwarf Green Curled	B.oleracea v.viridis L.	Northrup, King Seed Company
Kohl Rabi (KO) Early White Vienna	B.oleracea v.gongylodes L.	Northrup, King Seed Company
Mustard (MU) Tendergreen	B.nigra(L.)Koch.	Northrup, King Seed Company
Radish (RA) Long Scarlet Short Top	Raphanus sativus L.	Northrup, King Seed Company
Rape (RP) Dwarf Essex	B.napus L.	Johnson & Faris Seed Inc.
Rutabaga (RU) American Purple Top	B.napobrassica(L.)Mill.	Fredonia Seed Company
Turnip (TU) Purple Top White Globe	B.rapa L.	Northrup, King Seed Company

places (Table 3). Generally, the diseased leaves were placed in a moist chamber overnight before attempted isolation in order to stimulate sporulation. The lesions were examined under low magnification with a stereoscopic binocular microscope, and when abundant conidia were found, they were detached so as to fall onto the surface of hard-water agar in Petri dishes and left for 12 to 24 hours for germination. Isolated conidia were picked up from the surface of the agar with a sterile needle and placed on potato-dextrose agar and V-8 agar test tube slants. This method was the most successful in preventing contaminants and was used for each collection of all diseased plant materials. Cultures received from other places were re-cultured in Petri dishes on a V-8 agar medium. Spores were picked up with a needle, washed out in sterile water and poured onto the surface of water agar under aseptic condition. Single-spore isolations were made in the same way as with the diseased leaf isolations. All isolations from a given collection were identically symbolized and numbered corresponding to their hosts. For example, A. brassicae (Berk.) Sacc. was labeled A-1; A. brassicicola (Schw.) Wilt., A-2; A. raphani Groves and Skolko, A-3. The pathogens grew very well on potato-dextrose agar on which A. brassicae and A. brassicicola sporulated heavily. A. raphani sporulated poorly. Sporulation in culture was stimulated by wounding the mycelium by cutting the fungus covered agar into strips or square pieces. The majority of all isolates produced spores plentifully in V-8 agar without any special treatment. Spores were also obtained sparingly by culturing on water agar plates, although mycelium development was very poor. Fifty-five isolates were collected. Thirty-six isolates were from Gainesville, Florida, and nineteen isolates were from

TABLE 3

SOURCES OF ALTERNARIA BRASSICICOLA(SCHW.)WILT., A.BRASSICAE(BERK.)SACC., AND A.RAPHANI GROVES AND SKOLKO

Host Collected	Isolate No.* A1	A2	A3	Date Collected	Locality	Collection No.
Broccoli	1	1-2	-	1958-59	Gainesville,Fla.	
Broccoli	-	3-6	-	1959	N.C.State College	N-41-A, C,G,F
Cabbage	1-2	1-3, 7-9	-	1958-59	Gainesville,Fla.	
Cabbage	-	5	-	Dec.9,58	Hastings,Fla.	
Cabbage	-	10	-	Jun.2,59	Reddick,Ocala,Fla.	
Cabbage	-	4	-	Nov.21,58	Bradenton,Fla.	
Cabbage	-	11#	-	Jul.27,59	Bradenton,Fla.	S.C.246
Cabbage	-	6#	-	Dec.26,58	Everglades,Fla.	
Cabbage	-	12#	-	Sep.21,59	Denmark (Ottawa, Canada)	S-45 (seed)
Cabbage	-	13#	-	Oct.10,59	Univ.Arkansas	
Chinese Cabbage	1	1	-	1958-59	Gainesville,Fla.	
Chinese Cabbage	-	2	-	Dec.25,58	Bradenton,Fla.	
Collards	1-2	1-9, 12-13	-	1958-59	Gainesville,Fla.	
Collards	-	10-11#	-	Sep.Aug. 59	N.C.State College	N-41-K N-41-M
Caullflower	-	1	-	Nov.2,59	Gainesville,Fla.	
Mustard	1-2	1-4	-	1958-59	Gainesville,Fla.	
Radish	-	1#	1#	Sep.21,59	Winnipeg,Manitoba Ottawa,Canada.	56-81
Turnip	1	1-2	-	1958-59	Gainesville,Fla.	
Turnip	1	3#	-	Aug.12,59	N.C.State College	N-41-E
------	12250#	-	-	Jun.25,59	American Type	12250
		12251#	-	Jul.10,59	Culture Col-	12251
			13618#	Sep.1,59	lection	13618

*Indicating A1 : A.brassicae(Berk.)Sacc.
A2 : A.brassicicola(Schw.)Wilt.
A3 : A.raphani Groves and Skolko

#Indicating that the isolates were received as cultures.

other places (Table 3). These isolates were separated into three groups and different sub-groups by their host plant. There were forty-three isolates identified as A. brassicicola, ten and A. brassicae and two as A. raphani. Most isolates of A. brassicicola appeared to be very similar to others of that species. The septate mycelium was olive gray to grayish black. The spores developed abundantly in long chains in superficial, concentric zones. The isolations of A. brassicae were somewhat variable, but most were light brown to brownish gray with age. Spores were produced plentifully singly or in chains of two to three. The isolation of A. raphani on potato-dextrose agar appeared cottony, whitish to greenish gray or dark olive with age, and there was little or no sporulation. They grew more superficially on V-8 agar; there was plentiful sporulation in short chains of two to five. Chlamydospores were frequently found. One isolate from each sub-group was selected and preserved for further studies.

The isolates marked CO-12-A2, TU-1-A1, and 13618-A3 were used in all experiments reported upon in this paper as the representatives of Alternaria brassicicola, A. brassicae, and A. raphani, respectively.

THE DISEASES

Several common names have been applied to the diseases caused by Alternaria spp. on crucifers. Most of them are applied in regard to the symptoms, although some are names pertaining to the characteristics of the pathogen or its pathogenicity. The common names which have been frequently found in the United States and foreign countries (28, 51, 77, 81, 82, 83, 84) are as follows:

Alternaria brassicicola

Alternaria leaf-spot	- Weimer (1924), Weber (1932)
Black leaf-spot	- Harter & Jones (1918), Weimer (1924), Anderson et al. (1926), Weiss (1950)
Black mold	- Harter (1926)
Black spot	- Orton (1931)
Brown rot of cauliflower	- Weimer (1924)
Dark leaf-spot	- Gram and Weber (1953)
Leaf-blight	- Harter (1912)
Small silique mould	- Neergaard (1945)

Alternaria brassicae

Grey leaf-spot	- Weimer (1926), Anderson et al. (1926)
Large silique mould	- Neergaard (1945)
Macrosporium leaf-spot	- Taubenhaus (1918)

Alternaria raphani

Black pod blotch, seedling blight	- Weiss (1950)
Radish leaf-spot	- Walker (1952)
Stock silique mould	- Neergaard (1945)

In regard to the common names mentioned above, black leaf-spot, gray leaf-spot and radish leaf-spot are preferable and used herein.

Host Range

Many plants included in the Cruciferae have been reported to be attacked by each of the three species of Alternaria discussed here (51). One pathogen may cause more damage to a specific crop than the others under the same natural conditions. Crucifers grown commercially and used as a vegetable food, as listed on Table 1, with the exception of rape, which is used for animal feeding, have been found to be affected commonly by A. brassicicola and A. brassicae. A. raphani was found to cause more damage to radish and stock, Matthiola incana (L) R.Br. rather than to other hosts. In the United States, cabbage and cauliflower are the important crop plants which have been reported by a number of investigators to be infected by A. brassicicola in the field and during transit. The host range listed by Neergaard (51), Weber (81), Walker (77), Weiss (84), Groves and Skolko (29) and many others is as follows:

Common host of A. brassicicola, A. brassicae and A. raphani
Radish, Raphanus sativus (L.)

Common hosts of A. brassicicola and A. brassicae
Broccoli - Brassica oleracea v. botrytis L.
Brussel sprouts - B. oleracea v. gemmifera DC.
Cabbage - B. oleracea v. capitata L.
Chinese cabbage (Pe-tsai) - B. pekinensis (Lour.) Rupr.
and Pak-choi, B. chinensis L.
Collards - B. oleracea v. acephala DC.
Cauliflower - B. oleracea v. botrytis L.
Horse-radish - Amoracia lapathifolia Gilib
A. rusticana (Lam.) Gaertn.
Mustard (Leaf mustard) - B. juncea (L.) Coss.
and Black mustard - B. nigra (L.) Koch
Rape - B. napus L. and Bird rape, B. campestris L.
Rutabaga - B. napobrassica (L.) Mill.
Turnip - B. rapa L.

Common host of A. raphani
Stock, Matthiola incana (L.) R. Br.

Besides the hosts as given above, the pathogens were also found to attack some other hosts in nature. From positive results of inoculation tests done by many workers as listed by Neergaard (51) and Weiss (84) the host range may be extended as follows:

Corps infected by A. brassicicola and A. brassicae
- Crucifer species
  - Cheiranthus cheiri L. (Neergaard, 1945)
  - Iberis amara L. (Neergaard, 1945)
  - Matthiola incana (L.) R.Br. (Bolle, 1924)
- Other species
  - Godetia hybrida (Neergaard, 1945)
  - Lactuca sativa L. (Neergaard, 1945)

Crops infected by A. brassicicola
- Crucifer species
  - Aubrietia hybrida (Neergaard, 1945)
  - Capsella bursa-pastoris (L.) Medic. (Neergaard, 1945)
  - Crambe maritima (Neergaard, 1945)
  - Lunaria annua L. (Weiss, 1950; Baker & Davis, 1950)
- Other species
  - Andropogon sorghum (Young l.c.)
  - Avena sativa L. (Young l.c.)
  - Cucumis melo L. (Young l.c.)
  - Cucumis sativus L. (Neergaard, 1945)
  - Lycopersicum esculentum Mill (Young l.c.; Neergaard, 1945)
  - Phaseolus vulgaris L. (Bolle, Brinkman l.c.; Neergaard, 1945)
  - Pisum sativum L. (Young l.c.)
  - Triticum aestivum L. (Young l.c.)

Crops infected by A. brassicae
- Crucifer species
  - Brassica campestris v. sarsonii (Massee, 1906; Mason, 1908)
  - Bunias orientalis L. (Bolle, 1924)
  - Isatis tinctoria L. (Bolle, 1924)
  - Lepedium campestre (L.) R.Br. (Weiss, 1950)
  - L. sativum L. (Sydow, after Chupp & Pirone, 1935; Neergaard, 1937)
  - L. virginicum L. (Weiss, 1950)
  - Sinapsis (Brassica) alba L. (Clinton, 1904)
  - S. arvensis L. (found in Gainesville, Florida, as weeds, by the author, 1960)

Crops infected by A. raphani
- Crucifer species
  - Brassica chinensis L. (Yoshii, 1933)

B. oleracea v. capitata L. (Neergaard, 1945)
B. oleracea v. botrytis (Groves & Skolko, 1944)
B. spp. (Yoshii, 1933; Groves & Skolko, 1944)
Cheiranthus cheiri L. (Neergaard, 1945)
Iberis amara L. (Neergaard, 1945)

Other species
Lactuca sativa L. (Neergaard, 1945)

Many varieties of plants in other genera were inoculated artificially as listed by Neergaard (51) and Groves and Skolko (29), but infections did not occur.

History and Geographical Distribution

The history of the nomenclature of Alternaria brassicicola (Schw.) Wilt. and A. brassicae (Berk.) Sacc. is complicated. The binomial of A. brassicae has been applied to fungi producing both small or large muriform septa spore forms. This is undoubtedly the result of an incorrect interpretation of Saccardo's Sylloge. He mistook Macrosporium brassicae Berk. for A. brassicicola and gave the description of A. brassicae (Berk.) Sacc. as a small spore form. Owing to the confusion of these two species, the early statements, mostly before 1947 (77), concerning the occurrence of the diseases are very difficult to interpret correctly. In most reports of cabbage affected by Alternaria, it is evident from the symptoms described that the disease is caused by A. brassicicola.

Alternaria brassicicola (Schw.) Wilt.

The disease caused by this fungus was first found on cabbage in 1832 by Schweinitz (66). Diseased specimens were preserved in the herbarium at Kew labeled Helminthosporium brassicola L.v.S.Herb. Schw in Berkeley's handwriting. A spore drawing, presumably also by Berkeley,

accompanied the specimen. In 1880, Saccardo designated it as A. brassicae (Berk.) Sacc. var. minor Sacc., which was changed to A. brassicae (Berk.) Sacc. in 1886. He apparently was unaware that it was the same pathogen that he had listed as Helminthosporium brassicicolum Schw., and Macrosporium commune Rabenh. var. circinans (Berk. & Curt.) Sacc. Saccardo's statement has been followed by Voglino (76) in 1902; Ferraris (24), 1912; Weimer (82), 1924; Sewada (65), 1931; Weber (81), 1932; Yoshii (89), 1933; Fajardo and Palo (22), 1934; Rangel (60), 1945; Vestal (75), 1950, and Sherf (67), 1959. Different names were applied from time to time besides A. brassicae (Berk.) Sacc. as mentioned above; however, there are only three names commonly found in the literature at present.

Milbrath (48) described the fungus as Alternaria oleracea Milbrath in 1922 from leaves of cabbage, cauliflower, and broccoli. In 1924, Bolle (8) examined the type specimen of M. cheiranthi Fr. var. circinans Berk. and Curt. from Herb. Kew. 1875 labeling it as M. circinans Berk and Curt. (Bolle).

Wiltshire has proposed A. brassicicola (Schw.) Wilt. as the binomial for the small spored fungus. A. oleracea and A. circinans had been applied to it previously by Groves and Skolko (29) who considered that the valid name should be A. oleracea, even though Neergaard (51) believed it should be A. circinans. Wiltshire (86) in 1947 stated that the disease was found first by Schweinitz. By his examination of the co-type specimens which were preserved at Kew, and in Schweinitz's herbarium together with a photograph of the original herbarium packet which bore a rough drawing of two spores he concluded that the fungus described by Schweinitz was the same as A. oleracea or A. circinans. Schweinitz spelled

the specific name of his species "brassicola" and Saccardo corrected this to "brassicicolum," but Wiltshire proposed to substitute the name A. brassicicola (Schw.) comb. nov.

In Europe, Nielsen (54) stated that the fungus was the most destructive parasite on the cruciferous seed crops in Denmark. It was also found in Holland by Bolle and Doyer (51), in Italy by Goidanich (27), and in Sweden by Hammarlund (28). Neergaard (51) believed that this fungus was distributed over Europe by the infection of the cabbage seed. Mason (45) reported its occurrence on cabbage in Central Africa and India. It was first found in Ceylon by Bond (9) in 1947 on cabbage and kohlrabi. Yoshii (89), 1933, described its attack on cabbage in Japan and Su (71) mentioned that the fungus caused severe damping-off and leaf-spot in cabbage in Mandalay, Burma. In South America it was observed on both cabbage and cauliflower in Brazil by Puttemans (58), in Columbia by Toro (74), in Bolivia by Bell et al. (5), in El Salvador by Crandall et al. (16), and in Guatemala by Muller (50). In North America, Conners (15) reported it on cauliflower in Canada. In the United States the occurrence of black leaf-spot has been reported from thirty-five states. The disease has been reported annually in the Plant Disease Reporter and its supplements. It was reported on the outer leaves of cabbage or cauliflower doing damage in the fields or during transit. Through the channels of commerce dealing with seed, seedling and consumable produce, the disease is accounted for in all cabbage growing states referred to by Weber (81). In Florida in 1905 Rolfs (62) reported that a number of fields were completely ruined by this fungus. It was especially severe

on Jersey Wakefield and Bald head varieties. Burger (11) reported it on Chinese Cabbage collected near Gainesville.

Alternaria brassicae (Berk.) Sacc.

The fungus was first described in 1836 in England by Berkeley (6) as Macrosporium brassicae Berk. His description was incomplete. His statement, however, that sporidia were clavate, antennae form, 5-11 septate, rather longer than the peduncle, would be applied better to the long-beaked spored form, A. brassicae, than to a short or non-beaked spored form, A. brassicicola, as listed through a mistake of Saccardo. The name A. brassicae (Berk.) Sacc. has been frequently confused with the small spore form, A. herculea (Ell. and Mart.) Elliott, in the literature and in herbaria from 1917 until recently (84). Neergaard (51) reported that the disease was first reported by Kuhn in Germany in 1885 on rape and later by Raabe, Wollenweber and Raabe, and Pape. He included additional reports from other European countries, as in Italy by Peglion, Ferraris, Verona, Pollaci, and Goidanich; in Sweden by Ellasson, Hammarlund, and Bjoring; in Russia by Kikolna; in England by Brooks and Pethybridge; and in Holland by Ritzema and Bolle who pointed out that Saccardo's binomial was incorrect.) In Denmark it was found first by Rostrup in 1893 and later it has been mentioned in most of their annual reports of plant diseases. In Asia, Mason (45) observed the fungus in an herbarium in Pusa, India. Bond (9) found it on Chinese cabbage, Chinese mustard and horse radish in Ceylon. In Japan this disease and those caused by other Alternaria spp. on Cruciferae were studied by Yoshii (89). Fajardo and Palo (22) said that it caused serious leaf-spots on crucifers in the Philippine

islands. The fungus was also found by Arruda (2) to have attacked cauliflower in Brazil and by Litzenberger and Stevenson (42) in Nicaragua.

In North America, Conners (15) found it on broccoli in Canada. In the United States, gray leaf-spot is commonly found, accompanied to some extent with black leaf-spot, in all parts of the crucifer-growing areas, as stated by Weber (81). Stewart (70) reported that it was common on flat turnip and found occasionally on cabbage on Long Island. Weimer (82) mentioned it as occurring in the vegetation section near San Francisco. The disease has been reported also from Ohio by Chupp (12); from Florida by Weber (81) and Eddins (19); from seed producing areas in Skagit County, Washington, by Pound (57). Weiss (84) listed the disease as being found generally in the north from New York, Pennsylvania, New Jersey, Iowa, Indiana, Massachusetts, Utah, Oregon and California, but it was also common in the south in Arkansas, Georgia, Texas and Florida.

Alternaria raphani Groves and Skolko

The fungus was described and named by Groves and Skolko (29) in 1944. They isolated the fungus from radish seeds originating in Ontario, Quebec, British Columbia, and from seeds imported from the United States. According to Neergaard (51), the fungus was first reported in Denmark by Rostrup in 1894 under the name Macrosporium cheiranthi on stocks. Upon examination of herbarium material left in the Botanical Muslum in Copenhagen, he stated that the disease was due to A. raphani. In England, Ware (79) found it causing a leaf-spot disease on stocks in greenhouses. Neergaard mentioned that a sample of such leaves was examined by Wiltshire and determined by him to be identical with a species of Yoshii in

Japan described as A. brassicae (Berk.) Sacc. var. macrosporium, pathogenic on Chinese cabbage, radish and other species of Brassica. Neergaard in 1945 also found it on stock, radish and cabbage seeds, which he stated had been grown in or imported from Holland, Germany, Hungary, France or Denmark. The fungus was said by Groves and Skolko (29) to be present only on radish seed.

In the United States, radish seed from California, Michigan, Minnesota, New Jersey, Ohio and Pennsylvania has been found infected by A. raphani. Davis et al. (18) observed it in 1946 in a commercial field of garden stock grown for cut flowers in San Mateo County, California.

Economic Importance

The losses caused by these three parasites vary greatly from year to year, depending on the species of pathogens and the species of hosts attacked and upon other factors, such as the use of diseased-free seed, rotation of crops, careful handling during harvest, packing, transit and storage. Walker et al. (18) and Weimer (82) considered the disease in the field as of minor importance, not justifying any particular control measures. In contrast, Rolfs (62) reported cabbage completely ruined in a number of fields in Florida. The information was added by Weber (81) in 1932 that cabbage and cauliflower, both in the field and during transit, were the most affected, and it was destructive on radish, brussels sprouts, collards, broccoli, kohlrabi, turnips and Chinese cabbage. Groves and Skolko (29) reported turnip and Chinese cabbage as common hosts seriously affected by A. brassicae. Milbrath (48) reported considerable damage caused by black mold in San Francisco and San Mateo Counties, California,

in 1920. Chupp (13) also called attention to its severe damage to turnips in the field and in storage in Ohio. Davis (17) reported a serious outbreak of A. brassicae and A. brassicicola in Massachusetts in 1934. In 1941, Godfrey (26) reported severe losses of cabbage in the lower Rio Grande valley of Texas. One field was reported to have lost approximately $5,000 more than the previous year. Eddins and Burrell (20) stated that the disease, especially the one caused by A. brassicicola, together with downy mildew, caused greater losses in Hastings and Sanford, Florida, in the 1948-49 season than all other cabbage diseases combined. Eighty per cent of the cabbage seedlings were destroyed, and several fields were abandoned before cutting. Sherf (67) reported an outbreak of black leaf-spot throughout the cabbage growing areas in New York, ranging from Youngstown in northwestern New York to Riverhead, Long Island.

The exact loss to a crop resulting from leaf-spotting is very difficult to judge. During warm temperatures, heavy dew and moist seasons, plants in seedbeds may be killed or injured by Alternaria spot. Stands and yields may be reduced in the fields set with plants affected with the disease. In North America the disease has been reported annually since 1917 in the Plant Disease Reporter, causing considerable financial losses of cabbage and cauliflower and some other crucifer crops during transit or storage. Weimer (82) stated that the serious attacks on cauliflower by brown rot during transportation from the Pacific Coast to the eastern states often amounted to 100 per cent of curds damaged on arrival at their destination. From the market inspections of cabbage from

different states, one to four outer leaves were usually found infected. A very high percentage of damage has been reported in Plant Disease Reporter (1, 31, 32, 33, 41) which is given below.

State	No. of cars inspected	Percentage of infection
Alabama	2	100
California	2	80-87
Florida	10	75-100
	20	2-100
	11	35-65
Louisiana	3	45-100
Massachusetts	1	50
New York	41	50-90
Texas	26	50-100
Wisconsin	4	80

Since these three pathogens are seed-borne parasites (29, 38, 40, 77) losses may have occurred by lowering the yield of seed by reduced germination, by causing pre-emergence and post-emergence damping-off and in primary infection of the crop plants. Three to 4 per cent of the seed of crucifers were attacked by gray leaf-spot, according to the survey made by Neergaard (51) of cabbage seed growing areas during the period of 1935-1941. He found that 90 per cent of the plants were damaged by the black leaf-spot parasite. He decided that A. brassicae was a weaker parasite than A. brassicicola.

A. raphani was mentioned as a weak parasite. He assumed that with 15 to 25 per cent seed infection it might cause low germination. Samra (64) said that seed treatment for control of the disease on radish seed was needed in The Netherlands. Atkinson (3) reported that it was pathogenic on radish seed in all parts of Canada, but no estimation of losses has been found. In the United States, McLean (47) considered

A. raphani to be the most important factor causing low germination of radish seed in Michigan. The disease was also reported by Davis et al. (18) as causing complete destruction of several small plantings of stock grown for cut flowers in California.

Symptoms

Black and gray leaf-spot symptoms on crucifers have been described from time to time by many writers, including Rolfs (62), Fawcett (23), Milbrath (48), Burger (11), Weimer (82, 83), Toro (77), Weber (81), Ramsey et al. (59) and Eddins (19). Most of these descriptions were based on diseases on cabbage and cauliflower and the symptoms were often given collectively and briefly. Black and gray colored leaf lesions were observed to be produced by both pathogens. The colors of the lesions varied on the various hosts attacked. Under favorable conditions the sooty or dark colored appearance of the lesions caused by A. brassicicola resulted from its heavy sporulation in long spore chains. A. brassicae produced brown spots upon which were formed singly developed brown spores. Weimer (83) and Eddins (19) mentioned that the size and shape of spots and other characteristics of the diseases depended on the part of the plant affected and also on the temperature and humidity, whereas Milbrath (48) said that the characteristics of the spots depended largely upon the color of the leaves infected. Some confusion exists in the literature prior to 1947 dealing with these two pathogens which were frequently found infecting the same plant. Davis (17) found both beaked and non-beaked spores on Chinese cabbage and named the fungus A. brassicae f. pe-tsai. Information dealing with symptoms caused by A. brassicicola,

which has been listed incorrectly as A. brassicae, A. oleracea, and A. circinans, will be found more often than descriptions of the disease caused by other Alternaria spp. Rather satisfactory descriptions of the black leaf-spot are reported as a cause of transit and storage head-rot of cabbage, cauliflower and broccoli by Weimer (82), and Milbrath (48). Rangel (60) and Neergaard (51) mentioned the symptoms on cabbage seedlings and pods. In 1926, Weimer (83) described A. brassicae (Berk.) Sacc. as the cause of spots on leaves and floral parts of cauliflower. Weber (81) also gave descriptions of the diseases caused by the first two organisms on cabbage, cauliflower, and broccoli. He stated that the infections on turnip, radish, and Chinese cabbage are similar, except the spots on leaves enlarge more rapidly and the fungus produced fewer spores and caused the leaves to become yellow and die more rapidly. The symptoms on radish, stock, and wallflower caused by A. raphani were given briefly by Groves and Skolko (29), and more completely by Neergaard (51) and Atkinson (3). (See Table 4)

The symptoms of disease caused by A. brassicae on foliage of broccoli, brussels sprouts, cabbage, collard, cauliflower, kale, kohlrabi, rape and rutabaga are similar. These plants with thick waxy leaves are designated as the cabbage group of the crucifers. The production of young plants is often reduced by pre-emergence and post-emergence damping-off resulting from infected seed or contaminated soil. Seedlings frequently show cotyledonous infections after escaping disease earlier in their existence. These may prove fatal or be of little consequence, depending on whether high humidity is present. On older leaves the spots

TABLE 4

SHOWING SYMPTOMS OF THE DISEASE PRODUCED ON THIRTEEN CRUCIFERS BY THREE ALTERNARIA SPP.

Host	A. brassicicola	A. brassicae	A. raphani
Broccoli	Seedling - damping-off from soil borne fungus, also pre-emergence killing of seedlings. Early foliage - purplish spots 2 days after inoculation enlarge to 3 cm; tissue dries, causing distortion. Foliage - circular to oval, zonate, brown to sooty black spots that disintegrate at centers, copious sporulation. Petioles and stems - dark, sunken, elongated spots up to 5 cm, causing shedding. Flowers - stunting, distortion, killing of flowers; shriveling, brown color. Pods - small spots, distortion and shedding of pods.	Seedlings - damping-off, pre-emergence, killing of seedlings, seed carried. Early foliage - spots 4-5 days after inoculation, gray color, often severe. Foliage - spots 1.5 cm, smooth, circular dark brown, yellow margin. Petioles and stems - trace of infection. Flowers - trace Pods - none.	Seedlings - damping-off, pre-emergence, killing of seedlings, soil inoculum. Early foliage - small purplish spots at first develop into gray or black color, raised margins. Foliage - spots circular to oval up to 1 cm, persistent centers, yellow halo (Fig. 13). Petioles - rarely found lesions. Stems - rarely found lesions. Flowers - rarely found lesions. Pods - rarely found lesions.
Brussels sprouts	Similar to broccoli, aggregated lesions in the water holding areas of foliage. Spots surrounded by narrow yellow halo, sprouts stunted and malformed (Fig. 1).	Similar to broccoli, slightly darker gray margin and wider yellow halo.	Similar to broccoli, small, dark spots up to 3 mm, slightly raised margin, surrounded by translucent area and light chlorotic zone, weak parasite.

TABLE 4--Continued

Host	A. brassicicola	A. brassicae	A. raphani
Cabbage	Similar to broccoli, older lesions zonate, cracked, severe on petioles causing shedding and rot of head.	Similar to broccoli, spots on leaves light to dark gray, slightly corrugated, larger spots surrounded by wrinkling chlorotic area.	Similar to broccoli, spots up to 2 cm.
Chinese cabbage	Minute, purple spots turning brownish gray and enlarge rapidly up to 2.6 cm, circular to oval, zonate and disintegrating rapidly, some shedding (Fig. 2). Lesions on petioles and stem, 4-5 cm long, causing leaf breaking and shedding.	Purplish to black spots, enlarge up to 1.5 cm, circular to oval, concentric faint halo. Spots are light brown, abundant sporulation (Fig. 7). Petioles and stem lesions brown up to 4-5 cm, elongated spots on pods 1 mm sunken causing stunting of pre-matured opening.	Chlorotic spots becoming brown, bordering by dark gray and slightly raised margin surrounded by yellow area. Up to 5 mm.
Collards	Similar to broccoli, large spots on older leaves and few on the young ones.	Similar to broccoli, circular, zonate, 1.3 cm (Fig. 8).	Similar to broccoli, weak.
Cauliflower	Similar to broccoli, markedly zonate (Fig. 3).	Similar to broccoli, brown rot of flower, sporulation.	Similar to broccoli.
Kale	Similar to broccoli, leaf-spots dark gray to black disintegrated rapidly.	Similar to broccoli, small, purple becoming dark gray, light halo.	Similar to broccoli.
Kohlrabi	Similar to broccoli, few and elongated spots on petioles and stem.	Similar to broccoli, spots usually severe at the leaf-scars, enlarge up to 4-5 cm, irregular shape, zonate, bands, darker margin, and sunken	Similar to broccoli.

TABLE 4--Continued

Host	A. brassicicola	A. brassicae	A. raphani
Mustard - Similar to Chinese cabbage.		Similar to Chinese cabbage, thin, translucent spots with yellow halo, light color at the center instead of gray as on Chinese cabbage, zonate with darker brown zones alternately (Fig. 9).	Similar to Chinese cabbage (Fig. 14).
Radish - Similar to Chinese cabbage, brown, small spots with darker border, severe near the margin and distal end of the leaf (Fig. 4). Elongated spots on midribs and stem up to 3.5 cm.		Similar to Chinese cabbage, small spots, 5-8 mm, smooth without zonation, sharp demarcation between infected and healthy tissue. Yellow area on lower leaves. Shedding older leaves and crinkling of others (Fig. 10). Lesions on petioles and stem are frequently surrounded by purple color. The root above soil level became scabby.	Similar to broccoli, chlorotic spots 2 days after inoculation, turning brown to gray, darker margin bordering by yellow translucent area, 1 cm, round to irregular oval spots (Fig. 15). Stem lesions purplish. Root lesions scabby (Fig. 16).
Rape - Similar to broccoli, spots are surrounded by a pronounced yellow area (Fig 5).		Similar to broccoli, spots slightly surrounded by yellow zone (Fig. 11).	Similar to broccoli.
Rutabaga - Similar to broccoli.		Similar to broccoli.	Similar to broccoli.
Turnip - Similar to Chinese cabbage. Small, brown to gray spots up to 1 cm, frequently surrounded by purple halo or shot-hole appearance (Fig. 6).		Similar to Chinese cabbage, root infection has not been observed (Fig. 12).	Similar to Chinese cabbage.

Fig. 1.--Symptoms on the adaxial surface of brussels sprouts leaves infected by Alternaria brassicicola, left, 4 days after inoculation, the advanced stages of infection, right. X1/2.

Fig. 2.--Advanced stage of disease on the adaxial surface of a Chinese cabbage leaf infected by Alternaria brassicicola. The tip of the leaf was severely infected, and the dead tissue dropped. X1/2.

Fig. 3.--Advanced stage of disease on the adaxial surface of a cauliflower leaf infected with Alternaria brassicicola. Remarkably black, concentric ring, either cracked or not are shown. X1/2.

Fig. 1.--Symptoms on the adaxial surface of a radish leaf 7 days after inoculation with Alternaria brassicicola. Nat. size.

Fig. 5.--Advanced symptoms of disease on the adaxial surface of a rutabaga leaf infected by Alternaria brassicicola. X1/2.

Fig. 6.--Symptoms on the adaxial surface of a turnip leaf 4 days after inoculation with Alternaria brassicicola. Nat. size.

Fig. 7.--Advanced symptoms of disease on the adaxial surface of a Chinese cabbage leaf infected by Alternaria brassicae. X1/2.

Fig. 8.--Advanced symptoms on the adaxial surface of a collard leaf infected by Alternaria brassicae. X2/3.

Fig. 9.--Symptoms on the adaxial surface of a mustard leaf 12 days after inoculation with Alternaria brassicae. X1/2 (top); symptoms on seed pods and racemes (below), X2.

Fig. 10.--Symptoms on the adaxial surface of a radish leaf 9 days after inoculation with Alternaria brassicae. X2/3.

Fig. 11.--Symptoms on the adaxial surface of a rape leaf 12 days after inoculation with Alternaria brassicae. X2/3.

Fig. 12.—Symptoms on the adaxial surface of a turnip leaf 11 days after inoculation with Alternaria brassicae. X2/3.

Fig. 13.--Symptoms on the adaxial surface of a broccoli leaf 9 days after inoculation with Alternaria raphani. X1/2.

Fig. 14.--Symptoms on a section of the adaxial surface of a mustard leaf 18 days after inoculation with *Alternaria raphani*. X1/2.

Fig. 15.--Symptoms on the adaxial surface of a radish leaf 10 days after inoculation with Alternaria raphani. X2/3.

Fig. 16.--Infected radish root in advanced stage after inoculation with Alternaria raphani showing scabby lesions (right) compared with a check. X3/4.

enlarge slowly up to 1.5 cm in diameter. They are circular, light brown to dark gray in color, frequently show raised convex zonate corrugations surrounded by a rather sharp margin. The centers remain more or less intact, appearing to be thick and tough, and the surface becomes sparsely covered with brown conidiophores and conidia.

On Chinese cabbage, mustard, radish and turnip, the seedling infections are of the same nature as described above. These plants with thin non-waxy leaves are referred to as the mustard group. The leaf infections are brown, circular to oval, and enlarge rapidly up to 1.5 cm in diameter when scattered and remain small, less than 1 cm when plentiful.

On radish there is considerable variation from the above description, namely, the spots are usually much smaller and are almost black rather than brown and the centers of the spots remain more intact. Infections on the exposed areas of the roots of radish appear scabby. In the early stages of development there may be a purplish color on rutabaga and radish foliage.

The symptoms of disease caused by A. brassicicola on the seedlings of the plants of the cabbage group are indistinguishable from those described above. On the foliage the spots enlarge rapidly up to 3 cm if not crowded, otherwise many may coalesce to involve extensive leaf area. They are circular to oval, sunken dark brown turning to sooty black as spore production progresses. The centers become dry, frequently ridged, and occasionally crack. Disintegration of these areas does not generally develop. Lesions are commonly found on the main veins and petioles resulting in extensive damage to or killing of the leaf.

The diseases produced on seedlings of the mustard group are similar to the above descriptions. On the foliage of older plants, the lesions are circular up to 2.5 cm, brown to black, develop rapidly and the centers disintegrate particularly in damp weather, leaving a shot hole appearance. On radish, the lesions remain intact longer than on the other hosts of this group. Sometimes there is a definite purple tinge to the young lesions on radish and turnip leaves.

The symptoms of disease produced by A. raphani through inoculations in the greenhouse and out of doors on seedlings are similar to the above descriptions caused by the other Alternaria Spp. found on crucifers. On the plants of the cabbage and mustard groups the spots are small, black and sunken at first, gradually enlarging up to 3 mm in diameter. They are slightly raised, circular, dark gray, surrounded by a variable yellow halo. The centers remain intact whereas they fall out in the mustard group because of the thinner blade tissue. On radish, the leafspots become larger and the tissue remains intact longer than in the mustard group, but disintegrates under humid conditions. On exposed root areas, black lesions are formed that become rough and scabby.

The general distinguishing characteristics of the disease produced by A. brassicae are the brown spots and the production of many long, brown conidiophores and brown solitary conidia. For A. brassicicola the spots are larger in size and usually sooty black with abundant production of short, black conidiophores and black conidia in chains. A. raphani has not been found in nature on radish or any other crucifer in Florida. It could be identified tentatively by the yellow halo and the leaf lesions but would require spore examination for verification.

THE PATHOGENS

Taxonomy

The pathogens which cause gray leaf-spot, black leaf-spot, and radish leaf-spot on cruciferous plants are taxonomically placed in the genus Alternaria in the Section-Dictyosporae of the Family-Dematiaceae, Order-Moniliales, and Class-Fungi Imperfecti. In reviewing the literature, two genera, Alternaria and Macrosporium were mostly involved. The genus Alternaria was first described by Nees (53) in 1817, probably from A. tenuis and two years later Fries (51) described the genus Macrosporium, without mentioning the genus Alternaria. Both muriform-spore types were separated from each other by the production of catenulate spores by Alternaria (29, 37, 38, 56). At the present time, such a basis is found undependable. Elliott (21) pointed out that a more fundamental difference should be on the shape of spores. Planchon (56) reported great variations in Alternaria due to growth on different media. Elliott (21) found that all types of spores found in exsiccati or described in the Saccardo's Sylloge Fungorum under the genera Alternaria and Macrosporium produced conidial chains under favorable conditions. He stated that nearly all of Macrosporium spp. as listed by Saccardo belong to the genus Alternaria. The generic description of Alternaria Nees was proposed to be amended as follows:

Alternaria Nees. Conidiophores solitary or fasciculate, erect, or subdecumbent, simple or branched, generally short, colored. Conidia

muriform, often with few longitudinal septa, ovate, obclavate, or elongate, always with more or less definitely pointed apex, often long-beaked, colored, under favorable conditions forming chains. (Ex., A. tenuis, the type of the genus.)

There are several species and forms which have been reported found on cruciferous plant materials. Some species, such as A. tenuis sensu Elliott, and A. cucumerina (E. and E.) Elliott, were reported as weak parasites on cabbage (21). Neergaard (51) said that of the many varieties or forms which have been designated, hardly a single one has been retained. Varieties of A. brassicae such as A. brassicae v. dauci, A. brassicae v. citri, A. brassicae v. nigrescens have been raised to the specific rank as A. dauci, A. citri, A. cucumerina, respectively. This problem is not in the preview of this study.

A. brassicae (Berk.) Sacc., now known as the binomial for the large spore form was generally recognized by Saccardo as the small spore form until Bolle (8) pointed out the misunderstanding of Saccardo in 1924. She proposed A. brassicae (Berk.) Bolle for it and placed A. brassicae Sacc. among the synonyms of the small spore species. This made a new combination because her correction was not in accord with the International Rules. Neergaard (51), however, agreed with her. Elliott (21) classified it in 1917 as A. herculea (Ell. and Mart.) Elliott, which has usually been followed. Groves and Skolko (29), in 1944, objected to these names based on Article 61 and mentioned in Article 54 of the International Rules of Botanical Nomenclature which states that "When the specific epithet, on transference to another generic name, has been

applied erroneously in its new position to a different plant, the combination must be retained for the plant on which the epithet was originally based." Therefore, the specific name must be considered according to the type of the fungus, and this cannot be affected by the misinterpretation of Saccardo and his combination based on Berkeley's type. Therefore, the correct name should be A. brassicae (Berk.) Sacc. Wiltshire (86) and Walker (77) also agreed.

A. brassicicola (Schw.) Wilt., the small spore form, was encountered mostly in the literature as A. brassicae (Berk.) Sacc., because of the misinterpretation of Saccardo. A. brassicae (Berk.) Bolle has been misunderstood by the complication presented above; A. oleracea Milbrath was published as a new species in 1922 and is synonymous with A. brassicae as presented above; A. circinans (Berk and Curtis) Bolle, is also considered as a synonym, which Bolle (8) referred to as the correct name of this pathogen. A. oleracea was designated by her as a synonym of this fungus. Groves and Skolko (29) believed that A. oleracea should be the correct name according to Article 58 of the International Rules. A. brassicicola (Schw.) Wilt. was listed as the name of the small spored fungus by Wiltshire in 1947. He mentioned that this fungus was first found by Schweinitz in 1832, although he named it Helminthosporium brassicola. Wiltshire referred to Article 18 of the International Rules and credited to him as Alternaria brassicicola (Schw.) comb. nov. This name was accepted by Walker (77) and others, and it is well known in the literature at present.

A. raphani Groves and Skolko is the name of the third species of Alternaria which attacks crucifers. They reported it from Canada in 1944, whereas Neergaard (51) reported it as A. matthiolae sp. n. in Copenhagen in 1945. There is no doubt that the latter name is the synonym of the former according to the law of priority.

The synonyms and taxonomic descriptions of these three pathogens are as follows:

Alternaria brassicae (Berk.) Sacc.

Syn. : Listed by Neergaard (51) and Wiltshire (86).

Macrosporium brassicae Berk., 1836
Puccinia (?) brassicae Montagne, 1836
Sporidesmium exitiosum Kuhn, 1855
Rhopalidium brassicae Mont. and Fr., 1856
Polydesmus exitiosus (Kuhn) Kuhn, 1859
Cercospora bloxami Berk. and Br., 1882
Macrosporium herculeum Ellis and Martin, 1882
Cercospora lepidii Peck, 1884
Sporodesmium onnii Karst, 1891
Macrosporium brassicae Berk. var. macrospore Ellasson, 1897
Sporodesmium brassicae Massee, 1901
?Cercospora crassa Sacc. f. lunariae Ferraris, 1912
?Cercospora crassa Sacc. f. iberidis Ferraris, 1912
Both names listed by Neergaard, 1945, but Wiltshire found that Ferraris listed them without diagnosis.
?Leptosphaeria exitiosa (Kuhn) Rostrup, 1902
In doubt, no evidence of perithecial stage found on this species as mentioned by Rostrup.
Alternaria brassicae (Berk.) Sacc. var. exitiosa (Kuhn) Ferraris, 1912
Alternaria herculea (Ell. and Mart.) Elliott, 1917
Alternaria brassicae (Berk.) Bolle, 1924
Alternaria macrospora (Sacc.) Sawada, 1931
Alternaria exitiosa (Kuhn) Jorstad, 1945

On the leaves of various Cruciferae forming circular, zonate, light brown to grayish or dark brown spots, from less than 0.5 to 12 mm in diameter, sometimes coalescing; on the midribs of the leaves, spots oblong or linear, sunken; and on the heads of cauliflower it forms black spots.

Vegetative mycelium within the tissues, hyaline, 4 to 8 u in diameter; conidiophores amphigenous, arising in groups of 2 to 10 or more through the stomata, mostly toward the center of the spot, or in the concentric markings, with slightly swollen bases and rounded apices, smoke gray or grayish olive, seldom branched, straight or wavy, geniculate with a prominent scar at each geniculation, 14 to 171 u by 6 to 11 u and 0 to 7 septate; conidia, solitary or in chains of up to 4, obclavate, straight or slightly curved, light grayish olive to grayish olive, with a smooth (or inconspicuously warted) surface, 76 to 350 u by 11 to 42 u with the beak about one-third to one-half the length of the spore and 4 to 8 u in width, 6 to 19 cross septa, mostly 11 to 15, and 0 to 8 vertical or oblique septa, mostly 0 to 3.

Characteristic features of this species are: its light-brown thick conidiophores, the short chains of brown conidia, and the comparatively few cross septa, thick beaks, and smooth or almost smooth surface of the conidia (86).

Alternaria brassicicola (Schw.) Wilt.

Syn. : Listed by Neergaard (51) and Wiltshire (86).

Helminthosporium brassicola Schweinitz, 1832
Sporidesmium exitiosum Kuhn formae alternarioides and luxuriosum Kuhn, 1855
Polydesmus exitiosus (Kuhn) Kuhn formae alternarioides and luxuriosum Kuhn, 1855
Macrosporium chelranthi Fr. var circinans Berk. and Curt., 1875
Alternaria brassicae (Berk.) Sacc. var. minor Sacc., 1880
Macrosporium commune Rabenh. var. circinans (Berk. and Curt.) Sacc., 1886
Alternaria brassicae (Berk.) Sacc., 1886
Alternaria brassicae (Berk.) Sacc. var. microspora Brun., 1897
Helminthosporium brassicae P. Henn., 1902
Alternaria oleracea Milbrath, 1922
Alternaria circinans (Berk. and Curt.) Bolle, 1924
Alternaria brassicae (Berk.) Lindau, 1933

On the leaves of Cruciferae it forms dark brown to almost black, circular, zonate spots, from about 1 to 10 mm in diameter.

Vegetative mycelium in the tissues hyaline at first later brown or olivaceous, inter- and intracellular, 1.5 to 7.5 u in diameter; conidiophores amphigenous arising singly or in groups of 2 to 12 or more through the stomata, 0 to 3 septate, with the basal cell sometimes slightly swollen, olivaceous, rarely branched, straight and upright when solitary, often curled when fasciculate, not markly geniculate, usually with a single terminal scar, 20 to 70 u by 5 to 8 u; conidia borne in chains of 0 to 20 or more, sometimes branched, light to dark olivaceous, nearly cylindrical, oblong, usually tapering slightly toward the apex, or obclavate, the basal cell rounded, the beak usually almost non-existent, the apical cell being more or less rectangular or resembling a truncated cone, but occasionally better developed, short and thick, smooth or becoming slightly warted with age, 18 to 130 u by 8 to 30 u, with the beak about one-sixth the length of the spore and 6 to 8 u in width, with 1 to 11 mostly less than 6 cross septa, and usually few up to 6 longitudinal septa; a central pore is frequently visible in the cross walls.

The characteristic features of the species are the shape of the conidia, which is chiefly cylindrical to oblong with a poorly developed beak, and the predominantly smooth surface, and the readily visible pores in the cross walls (86).

Alternaria raphani Groves and Skolko

Syn. : Listed by Wiltshire (86)

Alternaria brassicae (Berk.) Sacc. var. macrospora Sacc., 1886
Alternaria matthiolae Neergaard, 1945

Colonies on malt agar effused, cottony, not zoned, reaching 6-8 cm in diameter in ten days, "pale olive gray" to "pale dull gray," occasionally darker to "olive gray" or "iron gray" (Ridgway), hyphae septate, branched, hyaline to smoky olive, 2-5-(7) u in diameter; chlamydospores numerous, olive brown, at first one-celled, round, finally many-celled and irregular in shape, conidiophores olive brown, septate, simple or sometimes branched, very variable in length, 4-7 u in diameter, usually enlarged toward tip; conidia catenulate in short chains, obclavate, beaked but the beaks usually short, smooth, olive brown, muriform, with 3-9 transverse septa and numerous longitudinal septa, constricted at the septa (40)-50-70-(94) x 15-25-(45) u on seedlings, on agar in Petri dishes (55)-70-115-(135) x 14-18-(25) u on inoculated leaves in the greenhouse; spots on seed pods, roughly circular, black, up to 4 mm in diameter.

Host : on aboveground parts of Raphanus sativus L. (29).

Morphology

No sexual stage of these three pathogens has been reported in the literature nor was one observed during these experiments. The characteristics and formations of mycelium, conidiophores, and conidia depend on factors such as temperature, hydrogen ion concentration, nutrition, and light. Other factors such as humidity and age of isolates, were also reported as the causes of variations (3, 51, 60, 82).

A. brassicae (Berk.) Sacc.

Colonies on various culture media range from light brown to dark gray in color, aerial or loose cottony, superficial or compact and felt-like, occasionally zonate growth was produced. The hyphal strands are 3.50-8.15 u in diameter, hyaline, branched, becoming dark gray, irregularly septate, and frequently moniliform on the main branches. Light pink chromogenic reaction was occasionally observed on colonies grown on potato-dextrose agar. Chromogenesis was also reported by Rangel (60) and Neergaard (51) to have occurred on rice and malt-extract agar. They varied greatly in spore production on potato-dextrose agar but generally the fungus copiously produced conidiophores and conidia in cultures.

Conidiophores on hosts are maculicole, epiphyllous, sparsely hypophyllous, and fasciculate. Neergaard (51) stated that they often projected from stomata. They are 4.50-10 u in diameter, 14.67 u - 73.75 u long, olivaceous to dark gray, straight or geniculate containing 1-3 conidial scars mostly close to the distal end, 2-6 septa, constricted at septa, usually unbranched. In cultures the length of conidiophores varies greatly. They are slightly constricted at septa, simple or branched, straight. Frequently they are found swollen at their apices (Fig. 17 and 18) and mostly show one conidial scar. A conidium develops by constriction of the distal part of the conidiophores (Fig. 17).

On the host conidia develop singly on the apex of a conidiophore; however, 2-4 conidia in a chain are commonly found in culture (Fig. 18) The characteristics of a series of conidial developments (Fig. 17) are similar to those of A. cucumerina (E. and E.) Elliott, as described by

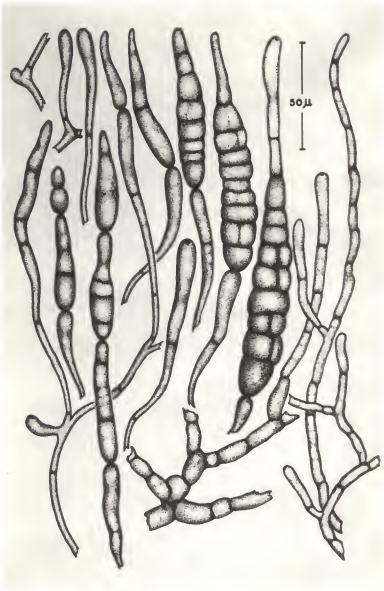

Fig. 17.--Camera lucida drawings showing Alternaria brassicae on potato-dextrose agar; mycelium, conidiophores, young conidia singly and in chains and muriform septations.

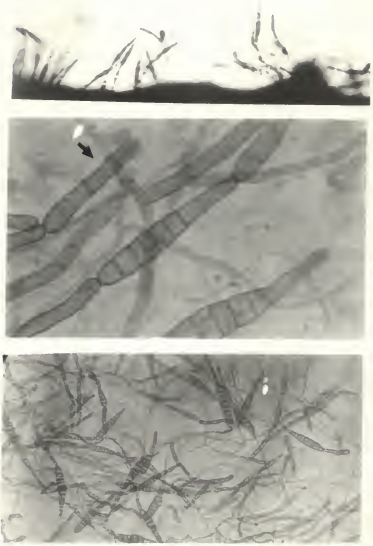

Fig. 18.--Conidiophores and conidia of Alternaria brassicae forming on a mustard leaf lesion (top, X40) and from potato-dextrose agar (middle and below, X430 and 100). The arrow indicates conidia spore formation.

Jackson (35). The formation of a conidium requires from 15 to 20 hours. The second and third conidia may be initiated at the distal end of the earlier conidium whether or not it has fully developed. No significant difference of catenulate conidia has been observed other than the length of supporting beaks which are often shorter than the distal one. Wiltshire (86) gave the length of beaks to be about 1/3 - 1/2 the length of the spore. Elliott (21) and Neergaard (51) called attention to the variations of size, shape, and septation concerning accurate characteristics and spore measurement.

The size of conidia which have been reported in the literature is shown on Table 5.

Conidia are dark olivaceous brown, obclavate, straight or slightly curved, gradually tapering toward the beak, muriform, slightly constricted at septa with regularly spaced transverse septations (Fig. 19). The surface is smooth; however, Elliott (21) stated that echinulation occurred in all of the species. Beaks are flexuous, slightly enlarged at the basal cells and occasionally at the distal end. They are hyaline and show 0-7 transverse septa. The lengths of beaks were sometimes about two times longer than usual. They were straight or curved, simple, and occasionally had an apical scar. Beak fragments germinate to produce mycelium and cause infection.

Measurements were made from one hundred spores from each collection. Isolates were grown on potato-dextrose agar. Septations that were viewed in the microscope in the uppermost plane and visible from both sides of the spore were counted as transverse septa. Septations which

TABLE 5

DATA ACCUMULATED FROM VARIOUS REPORTS SHOWING THE AUTHORS, HOSTS, SPORE MEASUREMENTS IN MICRONS AND SEPTATIONS PERTAINING TO ALTERNARIA BRASSICAE

Author	Source	Length of Spore	Width of Spore	No. of Septation	
				Trans-verse	Longi-tudinal
Ellis & Martin, 1882	Horse radish	200-225	21-26	many	few
Eliasson, 1897	Cabbage	115-240	20-25	6-11	-
Massee, 1901	B. campestris	160-200	25-35	-	-
Lindau, 1910		150-200	20-25	13	1-2
Bolle, 1924*	Cherry agar	90-350	14-42	7-19 (11-15)	0-8 (0-3)
Bolle, 1924*	-	92-204	13-30	-	-
Welmer, 1926	-	125-225	16-28	-	-
Yoshii, 1933	Brassica spp.	70-260	14-26	-	-
Chupp, 1936	Turnip & cultures	79.8-342	11.4-24.7	-	-
		143.6#	17.5#		
Goldanich, 1937	-	130-229	15-24	-	-
Groves & Skolko, 1944	Culture	100-238	16-33	15-12	few
Neergaard, 1945	Cultures	39-276	9-33	3-18	0-5
Wiltshire, 1947	-	76-350	11-42	6-19 (10-15)	0-8 (0-3)

*After Neergaard, 1945
#Average

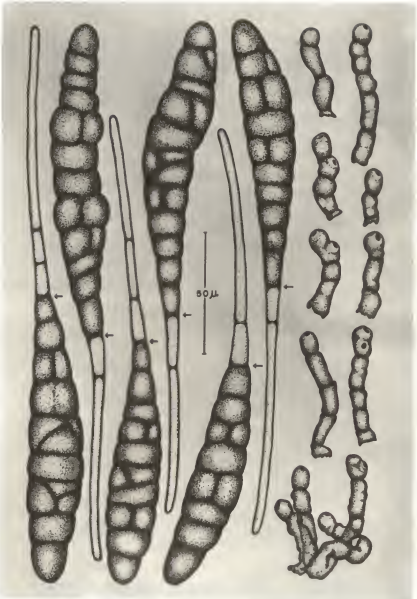

Fig. 13.--Camera lucida drawings of conidiophores and conidia of Alternaria brassicae. Arrows indicate the points of beak-body connection.

were continuous in approximately a straight line between two transverse septa were counted only once. The greatest overall diameter of the conidial body and the mid-point of the diameter of the beaks were measured.

Measurements of 100 conidia collected from mustard was made and compared with measurements of 100 spores obtained from V-8 agar medium, as shown in Table 6.

It is of interest to observe that the body of the spore was slightly longer and wider on the host than in culture and that the length of the beak on the host was longer than those produced in culture. The overall length of the spores produced on the host was greater than those produced in culture primarily because of added beak length.

Twenty-five conidia for measurements were collected at random from the lesions on collard, mustard, rutabaga and turnip in the field thirty days after inoculation. The results of the conidial measurements are shown in Table 7.

According to Table 7 the length of the body and beak varies slightly more than the body width. These variations are not significant and consequently the range sizes represented should make little difference in basic considerations for descriptions of this fungus.

The range of variations of the length and width of 200 conidia of this fungus is shown in graphic form (Fig. 20). The measurements of all conidia excepting 5 per cent are included in 120-210x14-32 u of the length and width dimensions.

TABLE 6

SHOWING COMPARATIVE MEASUREMENTS IN MICRONS OF THE BODIES, BEAKS, TOTAL LENGTHS, AND THE SEPTATIONS OF CONIDIA OF ALTERNARIA BRASSICAE PRODUCED IN ARTIFICIAL CULTURE AND ON MUSTARD

Measurement	In Culture	On Host
Spore body length	(44.0) 73.3-105.9 (146.7)	(55.4) 96.114.1 (141.8)
Spore body width	(11.4) 16.3-19.5 (31.0)	(14.7) 17.9-24.5 (32.6)
Transverse septa	(6) 10-11 (21)	(6) 10-11 (21)
Longitudinal septa	0-6	0-6
Spore beak length	17.9-122.2	(16-3) 45.6-65.2 (154.8)
Spore beak width	3.3-4.9	3.3-4.9
Total spore length	17.3-252.6	(86.4)148.3-184-2(252.6)

TABLE 7

SHOWING COMPARATIVE MEASUREMENTS IN MICRONS OF THE BODIES, BEAKS, TOTAL LENGTHS, AND THE SEPTATIONS OF CONIDIA OF ALTERNARIA BRASSICAE PRODUCED ON FOUR DIFFERENT HOSTS

Measurement	Collard	Mustard	Rutabaga	Turnip
Spore body length	92.2-154.8	76.6-154.8	73.4-150.0	47.3-138.6
Spore body width	17.9-29.3	17.9-32.6	17.9-31.0	16.3-27.7
Transverse septa	9-16	9-18	8-16	6-16
Longitudinal septa	1-4	1-6	0-5	0-6
Spore beak length	34.2-92.9	22.8-97.8	29.3-71.7	24.5-86.4
Total length	141.2-228.2	119.0-229.8	114.1-210.3	115.7-225.4

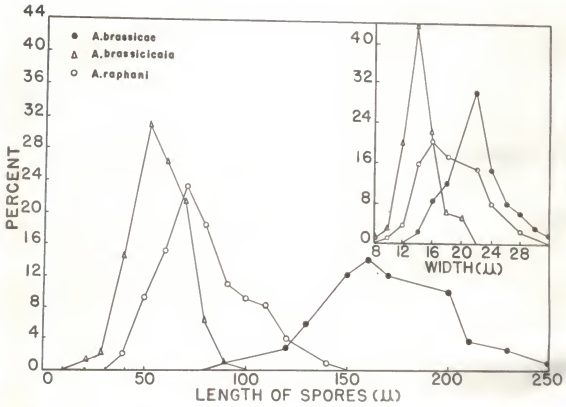

Fig. 20.--Graph showing the length and width of spores of Alternaria brassicae, A. brassicicola and A. raphani collected from host plants.

A. brassicicola (Schw.) Wilt.

The fungus grows well in various standard media. The colonies are suppressed and have sparse aerial growth, they are velvety to sooty in appearance, produced by the development of black conidiophores and conidia. The fungus regularly forms zonations of dark olive or lighter gray colors from the center of the colony of potato-dextrose agar outward with a white advancing margin. Milbrath (48) reported no visible zonation of the fungus grown on starchy and nitrogenous media. Neergaard (51) found similar characteristics of aerial mycelium of the isolates grown on standard nutrient agar.

The hyphae are 3.26-6.52 u in diameter, hyaline at first, becoming brown to dark olive with age, branched, septate, either constricted or not at septa, occasionally moniliform, usually submerged or appressed to the medium.

The conidiophores are maculicole, fasciculate or solitary, brown to dark olive, septate, constricted at septa, 4.50-6.75 u in diameter, 16.5-90.0 u long, straight or geniculate, containing 1-5 conidial scars. In culture conidiophores are variable in length and usually branched (Fig. 21). As a rule, the fungus sporulates abundantly and produces up to 10 conidia in chains.

The conidia of A. brassicicola are uniform and produced in long chains of about 8-12 conidia in culture and slightly shorter on host plants in the field. New conidia form at the apex of the former one. Lateral production is also common. On the conidia short knob-like side branches form from old conidia which are about 4.8-6.6 u wide and up to

Fig. 21.--Conidiophores and conidia of Alternaria brassicicola forming on a leaf lesion, X100, and in culture, X430. The arrow indicates secondary spore.

10.5 u long. Three lateral branches may occur on any conidium. The conidia are brown to dark olivaceous, smooth, obclavate, conical or cylindrical to oblong and only slightly tapering toward the apices, and are terminated with short, lighter brown, truncated apical cells (Fig. 22). The beaked forms are reported by Neergaard (51) whereas Wiltshire (86) referred to them as having a poorly developed beak about one-sixth of the conidial length or about 6 to 8 u width. Elliott (21) and Bourgin (10) mentioned that the echinulation or papilation on the surface of spores was sometimes observed. The size of conidia which have been reported in the literature is shown on Table 8.

One hundred conidia collected from potato-dextrose agar medium and one hundred from cabbage plants in the field were measured, as shown on Table 9.

The variations of length and width of conidia from host plants were given in graphic form (Fig. 20).

The measurements of conidia from culture and host vary only slightly, the size of conidia in culture is smaller than those from the host plants.

A. raphani Groves and Skolko

The characteristics of the fungus colonies on potato-dextrose agar culture media in Petri dishes varied from creamy white to smoky gray on the upper surface. They were black on the under surface. The mycelial weft was flat to aerial, thin to cottony. Dark gray radiations and slight zonations were occasionally observed. Atkinson (3) observed great variations of mycelial growth, colors and the presence or absence of

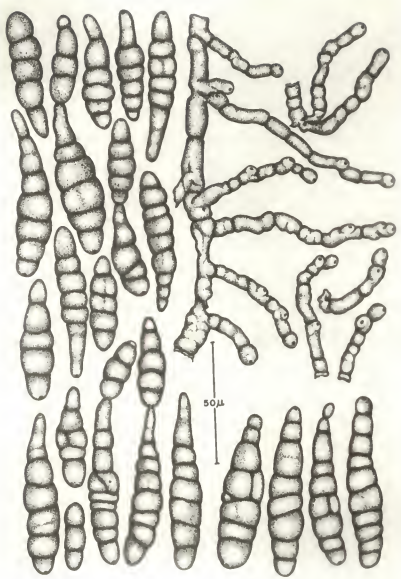

Fig. 22.--Camera lucida drawings of conidiophores and conidia of Alternaria brassicicola from host material.

TABLE 8

DATA ACCUMULATED FROM VARIOUS REPORTS SHOWING THE AUTHOR, HOSTS, SPORE MEASUREMENTS IN MICRONS AND SEPTATIONS PERTAINING TO ALTERNARIA BRASSICICOLA

Author	Source	Length of Spore	Width of Spore	No. of Septation	
				Trans-verse	Longi-tudinal
Saccardo, 1886	Brassica sp.	50-60	12-14	5-11	-
Massee, 1893	-	50-85	12-18	-	-
Elliott, 1917	Cabbage	13-59	5.0-19.0	-	-
Elliott, 1917	Cultures	8-51	5.0-17.0	-	-
Stevens, 1919	-	60-80	14-18	6-8	-
Milbrath, 1922	Cabbage	29.7-61.2 (43.7)#	8.9-12.3 (10.5)#	1-7	0-few
Milbrath, 1922	Cultures	13.4-70	6.5-14.0	1-9	0-few
Welmer, 1924	Cabbage & Cultures	11-17	6.5-16.8	1-10	0-few
Bolle, 1924*	Cabbage & Cultures	29-108	8.0-25.0	3-11	0-5
Yoshii, 1933	Cabbage & Cultures	15-86	8.0-22.0	-	-
Lindau, 1933		11.5-44	7-9	-	-
Groves & Skolko, 1944	Cultures	20-80 (30-50)	10-18 (10-15)	3-10 (3-7)	0-few
Neergaard, 1945	Cultures	7.5-65.5	3-19	1-9	0-few
Wiltshire, 1947	-	18-130	8-30	1-11 (6)	0-6

*After Neergaard, 1945.
#Average.

TABLE 9

SHOWING COMPARATIVE MEASUREMENTS IN MICRONS OF THE BODIES, BEAKS, TOTAL LENGTHS, AND SEPTATIONS OF CONIDIA OF ALTERNARIA BRASSICICOLA PRODUCED IN ARTIFICIAL CULTURE AND ON CABBAGE

Measurement	In Culture			On Host		
Spore body length	(13.3)	24.5-35.0	(52.5)	(14.7)	45.6-55.4	(86.4)
Spore body width	(6.5)	10.0-12.0	(16.1)	(8.2)	11.4-16.3	(19.6)
Transverse septa	(1)	3-5	(6)	(2)	5-8	(11)
Longitudinal septa		0-5			0-4	
Spore beak length		none			none	
Spore beak width		none			none	
Total spore length	(13.3)	24.5-35.0	(52.5)	(14.7)	45.6-55.4	(86.4)

zonation. No zonation was found by Groves and Skolko (29). The pathogen usually formed spores sparsely on the culture media, but chlamydospores were produced abundantly.

The hyphae are 3.26-6.52 u in diameter, hyaline at first becoming brown greenish gray to dark olive with age, branched, irregularly septate, with slight to bead-like constrictions at the septa. The old mycelium tends to curl, enlarge and form chlamydospores.

Chlamydospores were produced abundantly in all culture media within a few days. They may be formed aerially, on the surface of the medium or submerged. They arose first as single, thick-walled, globose to oblong intercalary cells (Fig. 23).

Conidiophores on natural hosts are maculicole and epiphyllous as were those produced by the other pathogens studied. They are 4.5-7.5 u in diameter, 29.34-159.74 u long, solitary or fasciculate, brown to dark olive, straight or curved, septate, slightly constricted at septa, containing 1-2 conidial scars near the apex. In cultures they varied greatly in length and usually formed laterally on the hyphae.

The conidia of A. raphani are formed in short chains. On host plants they may appear singly or 2-3 in a chain. Sporulation in culture media usually is not abundant. The conidia may be formed singly or in chains of 2-6 and rarely contain lateral branches. Atkinson (3) observed two types of spores, the large typical and small atypical ones. No measurement or descriptions of those types were given. He also mentioned that media containing yeast extract supported the best sporulation. Abundant spores of beaked and beakless forms (Fig. 23) were found produced in most leaf decoction agar media.

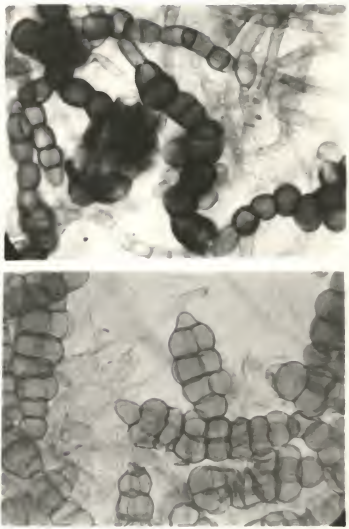

Fig. 23.--Chlamydospores and spores of Alternaria raphani in culture. X430.

The conidia are obclavate to irregular oval, tapering toward the beaks, which are short and hyaline. The surface is smooth and constricted at the septa (Fig. 24). They become brown to dark olive with age. The size of conidia as mentioned in the literature is shown in Table 10.

One hundred conidia grown on kohlrabi leaf decoction agar and one hundred conidia from radish leaves were measured. The results of the measurement of conidia from culture are shown in Table 11.

There appears to be only slight variation in these measurements, none of which are significant. The variations of length and width of conidia from host plants are given in graphic form (Fig. 20).

The following descriptions are considered as the criteria to differentiate them.

1. The thallus - A. brassicicola in culture is dark, sooty to low velvety with a white band margin, and obviously zonate. The colony of A. brassicae is gray to light brown in color toward the margin without obvious zonation, aerial mycelium rarely occurs and colonies are thin. A. raphani is usually whiter or creamy to brownish gray on the top surface and dark to nearly black below. When the colony is flat, the color becomes light brown rarely dark, chlamydospores are seen sparsely.

2. The spores - A. brassicicola spores from the host or from cultures are smaller than those of the other two species; they are dark, forming in long chains, cylindrical to oblong, have short beaks or no beaks, and have few transverse and longitudinal septations. The spores of A. brassicae are largest; they usually form singly, and have many transverse septa and several longitudinal septations; they are light in

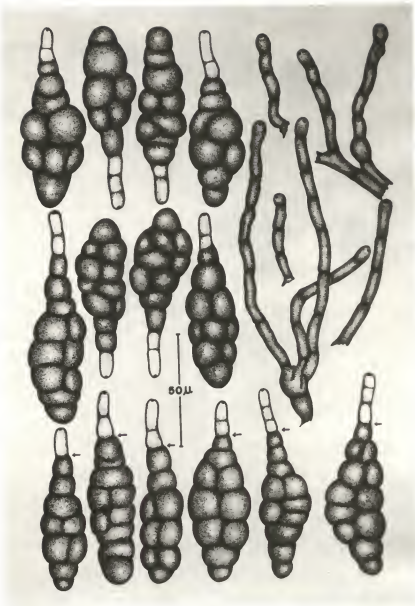

Fig. 24.--Camera lucida drawings of conidiophores and conidia from Alternaria radicali from host material. Arrows indicate the points of beak-body conjunction.

TABLE 10

DATA ACCUMULATED FROM VARIOUS REPORTS SHOWING THE AUTHORS, HOSTS, SPORE MEASUREMENTS IN MICRONS AND SEPTATIONS PERTAINING TO ALTERNARIA RAPHANI

Author	Source	Length of Spore u	Width of Spore u	No. of Septation Transverse	No. of Septation Longitudinal
Saccardo, 1886	Cabbage	120-140	20-25	6-8	-
Yoshii, 1933	Chinese Cabbage				
	Related spp.	60-150	10-23	-	-
Ware, 1936	Stock	30-140 (86)	12-24 (18)	2-14	-
Groves & Skolko, 1944	Radish	55-135 (70-115)	14-25 (14-18)	3-9	Numerous
	Seedlings on agar	40-94 (50-70)	15-45 (15-25)	3-9	Numerous
Neergaard	Cabbage, Stock, Radish and Cultures	27-123 (body: 12-78, beak: 3-76.5, beak: 0.7 transverse septa)	7.5-31.5	2-10	0-10

TABLE II

SHOWING COMPARATIVE MEASUREMENTS IN MICRONS OF BODIES, BEAKS, TOTAL LENGTHS AND SEPTATIONS OF CONIDIA OF ALTERNARIA RAPHANI PRODUCED IN ARTIFICIAL CULTURE AND ON RADISH

Measurement	In Culture			On Host		
Spore body length	(28.7)	45.6-55.4	(88)	(24.4)	45.6-58.7	(114.1)
Spore body width	(11.9)	15.4-25.2	(44.0)	(9.8	13.0-21.2	(29.3)
Transverse septa	(3)	5-7	(10)	(2)	6-9	(12)
Longitudinal septa	(0)	3-4	(8)	(0)	3-6	(12)
Spore beak length	(0)	19.6	(24.5)	(0)	10.5-25.1	(4.24)
Spore beak width		none			none	
Total spore length	(30.8)	45.6-75.0	(95.3)	(40.7)	60.3-83.1	(141.8)

color, obclavate, have slight constrictions at the septa, and have long thick beaks. The spores of A. raphani are smaller than those of A. brassicae. In length they approach the size of those of A. brassicicola. They may appear singly or in chains up to six. They are broader, contain numerous longitudinal septations, and have shorter beaks than those of A. brassicae. The spores are dark in color, and seem to be oval-obclavate rather than oblong; they are irregular and are markedly constricted at the septa.

Physiology

Growth on Various Media

Alternaria brassicae, A. brassicicola and A. raphani grow well in artificial culture media. There are considerable differences in their rates of development and sporulation, influenced not only by the species of the pathogens but also by the source of nutrition. Frequently A. brassicae (60) and A. raphani (3) have been reported to sporulate poorly on potato-dextrose agar as compared with sporulation of A. brassicicola on such medium. Malt extract agar and standard nutrient agar were used by Neergaard (51) for growing these parasites. He got positive results in sporulation of A. brassicae and A. brassicicola but only extreme mycelial development of A. raphani. Atkinson (3), however, found little or no sporulation on media other than potato-dextrose agar or malt agar. He believed that radish plants contained the essential substances for sporulation, but he failed to obtain spores by using potato-dextrose agar containing mineral ash of radish seed. Different nutrient liquid media composed of the basal glucose-nitrate salts, amino acids, growth factors,

yeast extract, and their combinations were tested by him, and he reported that the variation of spore production was correlated with the strains of the fungus rather than the nutrients of the medium. Media containing yeast extract allowed for maximum sporulation in some strains.

An experiment was conducted to determine the influence of various media on vegetative growth and sporulation under the same environmental conditions. Twenty-one solid media were used, including Difco agar media, one sweet potato agar medium and thirteen different cruciferous leaf decoction agar media prepared as indicated below.

A. Difco bean pod agar. Prepared by recipe on the label. pH 4.8

B. Difco corn meal agar. Prepared by recipe on the label. pH 5.2

C. Difco Czapek agar. Prepared from Difco Czapek liquid medium by adding 2 per cent Difco powder agar. pH 6.5

D. Difco lima bean agar. Prepared by recipe on the label. pH 6.8

E. Difco potato-dextrose agar. Prepared by recipe on the label. pH 5.2

F. Difco prune agar. Prepared by recipe on the label. pH 5.0

G. Sweet potato agar. Prepared by extracting 250 g of sweet potato root in 1000 ml distilled water then adding 20 g of agar. pH 5.3

H. V-8 agar. Prepared according to the formula of Miller (49). pH 5.9

I. Broccoli agar. Prepared as number G, avoiding mid vein. pH 5.2

J. Brussels sprouts agar. Prepared as number G. pH 5.4

K. Cabbage agar. Prepared as number G. pH 5.3

L. Chinese cabbage agar. Prepared as number G. pH 5.4

M. Collards agar. Prepared as number G. pH 5.4

N. Cauliflower agar. Prepared as number G. pH 5.2

O. Kale agar. Prepared as number G. pH 5.4

P. Kohlrabi agar. Prepared as number G. pH 5.5

Q. Mustard agar. Prepared as number G. pH 5.2

R. Radish agar. Prepared as number G. pH 5.3

S. Rape agar. Prepared as number G. pH 5.3

T. Rutabaga agar. Prepared as number G. pH 5.2

U. Turnip agar. Prepared as number G. pH 5.4

A 3-millimeter disc of each pathogen was transferred to the center of each Petri dish containing 15 ml of the medium. Three replications were made and the culture dishes were incubated at 20°C. The rate of growth was measured by taking two diameter readings at right angles to each other daily for nine days, being terminated when certain colonies had extended to the edges of their Petri dishes. Frequently the fungus growth was so thin that the margins of colonies were difficult to detect without the aid of a hand lens. Consideration was given to the possibility of determining the dry weight of the thallus as a means of obtaining supplemental data that would verify the accuracy of the measurements. This procedure was abandoned, however, due to the lack of facilities for making such determinations accurately. The data acquired from these experiments are shown in Tables 12, 13, 14, 15, 16 and 17 and in graphic form (Fig. 25) for A. brassicae, A. brassicicola and A. raphani respectively, as designated.

The data obtained in the preceding experiments concerning the growth characteristics of A. brassicae, A. brassicicola and A. raphani indicate that these three fungi were capable of growing and producing spores on a wide range of media and at the same time developed in such distinctive patterns that when compared with each other, distinctive features were manifested. There was no medium that was outstandingly favored

TABLE 12

DESCRIBING GROWTH CHARACTERS OF *ALTERNARIA BRASSICAE* ON VARIOUS HARD AGAR MEDIA AFTER NINE DAYS AT 20°C

Medium	Diameter in cm	Sporulation	Other Characteristics of Colonies
A. Difco bean pod agar pH 5.7	4.59	Abundant, brown spores single or in chains of 2	Thin, superficial, curly growth, frayed margin, colorless to brownish gray
B. Difco corn meal agar pH 5.7	2.45	Abundant, light to dark brown, single or in chains of 2-3	Very thin, superficial, frayed, curly, colorless to dark brown
C. Difco Czapek agar pH 7.3	3.94	Abundant, brown, single or in chains of 2-3	Thin, superficial, curly, submerged, colorless to brown or gray growth
D. Difco lima bean pH 7.2	4.52	Abundant, brown, spores mostly single	Very thin edge, frayed, colorless to light gray, superficial slightly thicker near the center
E. Difco potato-dextrose agar pH 5.9	3.07	Abundant, brown to gray, single or in chains of 2-3	Very thin, colorless to brown or gray, superficial, frayed, curly growth in medium
F. Difco prune agar pH 5.7	2.30	Abundant, single or in chains of 2-3	Very thin, superficial, frayed, no curly growth, brown to gray
G. Sweet potato agar pH 6.3	2.95	Abundant, brownish-gray, single or in chains of 2-3	Thin, superficial, curly to clumped moniliform mycelium
H. V-8 agar pH 7.3	3.47	Abundant, single or in chains of 2 rarely 3	Thin, superficial, thicker near the center, light brown to light gray

TABLE 12--Continued

Medium	Diameter in cm	Sporulation	Other Characteristics of Colonies
I. Broccoli leaf agar pH 6.0	3.35	Abundant, single or in chains of 2-3	Thin, superficial, frayed, light brown to dark brown or gray, conidial patch slightly aerial near the center
J. Brussels sprouts leaf agar pH 6.3	3.84	Abundant near the center	Thin, superficial, frayed, light brown to gray
K. Cabbage leaf agar pH 6.7	3.65	Abundant near the center	Thin, superficial, brown to gray patches
L. Chinese cabbage leaf agar pH 6.8	4.30	Abundant near the center	Very thin, slightly frayed, superficial, brown to gray near the center
M. Collard leaf agar pH 6.5	4.04	Abundant, dark gray patches	Thin, superficial, frayed, whitish to brown, cottony near the center, curly mycelium
N. Caulif. leaf agar pH 6.4	4.65	Abundant, single or in chains of 2-3	Thin, superficial, frayed, whitish brown to brownish gray
O. Kale leaf agar pH 6.7	4.49	Abundant	Thin, superficial, frayed, brownish gray, slightly zonated
P. Kohlrabi leaf agar pH 7.0	4.14	Abundant	Thin, superficial, frayed brownish gray, not zonated
Q. Mustard leaf agar pH 6.0	3.54	Abundant, single or in chains of 2-3	Thin, superficial, frayed, brown to dark gray patches

TABLE 12--Continued

Medium	Diameter in cm	Sporulation	Other Characteristics of Colonies
R. Radish leaf agar pH 5.9	3.13	Abundant, single or in chains of 2-3	Thin, superficial, small oil drops observed, dark gray
S. Rape leaf agar pH 6.3	2.75	Scattered spores near the center	Very thin, superficial growth, frayed
T. Rutabaga leaf agar pH 6.2	2.57	Scattered spores near the center	Very thin, abundant oil drops found along the mycelium
U. Turnip leaf agar pH 6.8	3.12	Abundant	Thin, superficial, frayed, brown to light gray

Sporulation was generally abundant on each of the media. In most instances the conidiophores and conidia were of a light brown to gray color and the spores developed in a catenulate manner. The mycelium was superficial in most cases, rather thin and of a solid or patchy brown color. In general the appearance of the cultures was very similar in most respects. They would normally be considered the same species of fungus even though certain ones showed retarded growth compared with the best developed colonies.

TABLE 13

DIAMETER OF COLONIES OF ALTERNARIA BRASSICAE IN CM, GROWN AT 20°C FOR NINE DAYS ON VARIOUS HARD AGAR MEDIA

Agar Medium*	Diameter of Colonies on Indicated Days								
	1	2	3	4	5	6	7	8	9
A. Difco bean pod	.45	.85	1.70	2.25	2.70	3.25	.350	3.70	4.53
B. Difco corn meal	.40	.65	.93	1.20	1.40	1.73	1.98	2.18	2.45
C. Difco Czapek	.42	.80	1.46	2.22	2.77	2.95	3.23	3.62	3.94
D. Difco lima bean	.50	.80	1.32	1.94	2.50	3.09	3.32	3.98	4.52
E. Difco pot.-dext.	.50	.83	1.19	1.50	1.70	2.07	2.30	2.62	3.07
F. Difco prune	.40	.67	.99	1.09	1.15	1.65	1.89	2.00	2.30
G. Sweet potato	.43	.80	1.10	1.37	1.60	1.85	2.40	2.71	2.95
H. V-8	.55	1.00	1.25	1.54	1.97	2.35	1.72	3.10	3.47
I. Broccoli leaf	.57	.96	1.46	1.89	2.25	2.54	2.78	3.10	3.35
J. Brussels spr.	.59	.99	1.59	1.97	2.40	2.89	3.25	3.56	3.84
K. Cabbage leaf	.64	1.07	1.69	2.02	2.42	2.74	3.00	3.32	3.65
L. Chinese cab.	.53	.92	1.59	2.17	2.75	3.35	3.78	4.04	4.30
M. Collard leaf	.52	.88	1.50	2.15	2.67	3.17	3.44	3.78	4.04
N. Caulif. leaf	.57	.99	1.65	2.20	2.77	3.30	3.72	4.10	4.65
O. Kale leaf	.57	1.32	2.14	2.64	3.06	3.47	3.75	4.09	4.49
P. Kohlrabi leaf	.57	1.27	1.99	2.44	2.98	3.35	3.67	3.89	4.14
Q. Mustard leaf	.50	.77	1.22	1.62	1.92	2.35	2.64	2.87	3.54
R. Radish leaf	.50	1.15	1.35	1.75	2.15	2.53	2.72	2.94	3.13
S. Rape leaf	.50	.87	1.37	1.58	1.82	2.20	2.53	2.64	2.75
T. Rutabaga leaf		.40	.65	.85	1.27	1.43	1.88	2.22	2.47
U. Turnip leaf	.43	.68	.95	1.06	1.47	2.13	2.60	2.73	3.12

*Capital letters in front of the media correspond to those of Figure 25.

These data indicate that Difco bean pod agar and the media prepared from foliage of Chinese cabbage, collards, cauliflower, kale and kohlrabi were most favorable sources of nutrition for this fungus with cauliflower being the best. The poorest growth was produced on Difco corn meal, Difco prune, sweet potato, rape, and rutabaga agars with Difco prune agar producing the poorest growth.

TABLE 14

DESCRIBING GROWTH CHARACTERS OF *ALTERNARIA BRASSICICOLA* ON VARIOUS HARD AGAR MEDIA AFTER NINE DAYS AT 20°C

Medium	Diameter in cm	Sporulation	Other Characteristics of Colonies
A. Difco bean pod agar pH 5.7	6.79	Abundant, dark, in long chains of 8-10	Superficial, low velvety, concentric rings of dark brown and olive gray, light brown margin
B. Difco corn meal agar pH 5.7	5.93	Abundant in long chains of 8-10	Clump, slightly aerial, concentric, brown
C. Difco Czapek agar pH 7.3	8.10	Abundant, in long chains	Grayish black or sooty, velvety, concentric
D. Difco lima bean agar pH 7.2	7.29	Abundant toward the margin	Superficial, brownish to olive gray, velvety, concentric
E. Difco potato-dextrose agar pH 5.9	7.39	Abundant, sooty, long chains	Superficial, velvety, concentric, olive black
F. Difco prune agar pH 5.7	6.57	Abundant, long chains	Superficial, brownish to olive gray, velvety, concentric
G. Sweet potato agar pH 6.3	8.22	Abundant, long chains	Superficial, velvety, olive black, concentric
H. V-8 agar pH 7.3	8.35	Abundant, long chains	Superficial, velvety, olive black, concentric
I. Broccoli leaf agar pH 6.0	8.28	Abundant, long chains	Superficial, velvety, olive black, sharply concentric

TABLE 14--Continued

Medium	Diameter in cm	Sporulation	Other Characteristics of Colonies
J. Brussels sprouts leaf agar pH 6.3	7.50	Abundant, long chains	Superficial, brownish to olive gray, velvety, sharply concentric
K. Cabbage leaf agar pH 6.7	8.54	Abundant, long chains	Similar to G, lighter concentric
L. Chinese cabbage leaf agar pH 6.8	8.27	Abundant, long chains	Similar to G, light concentric
M. Collards leaf agar pH 6.5	8.28	Abundant, long chains	Similar to G, concentric
N. Caulif. leaf agar pH 6.4	8.18	Abundant, long chains	Similar to G, sharp concentric
O. Kale leaf agar pH 6.7	8.57	Abundant, long chains	Similar to G, concentric
P. Kohlrabi leaf agar pH 7.0	8.48	Abundant, long chains	Similar to G, sharp concentric
Q. Mustard leaf agar pH 6.0	7.22	Abundant, long chains	Similar to D, sharp concentric
R. Radish leaf agar pH 5.9	7.88	Abundant, chains	Similar to D, light concentric
S. Rape leaf agar pH 6.3	7.85	Abundant, long chains	Similar to G, very sharply concentric

TABLE 14--Continued

Medium	Diameter in cm	Sporulation	Other Characteristics of Colonies
T. Rutabaga leaf agar pH 6.2	8.05	Abundant, long chains	Similar to G, sharply concentric
U. Turnip leaf agar pH 6.8	7.32	Abundant, chains	Similar to D, concentric

The growth characters of A. brassicicola on these media were astonishingly similar and uniform. Sporulation was evident in a characteristic fashion in every instance and other features were exceedingly comparable. The media was in all cases sufficient to produce uniformly typical growth of the fungus.

TABLE 15

DIAMETER OF COLONIES OF ALTERNARIA BRASSICICOLA IN CM GROWN AT 20°C FOR NINE DAYS ON VARIOUS HARD AGAR MEDIA

Agar Medium*	Diameter of Colonies on Indicated Days								
	1	2	3	4	5	6	7	8	9
A. Difco bean pod	.70	1.34	2.25	2.99	3.74	4.30	5.05	5.77	6.79
B. Difco corn meal	.70	1.27	2.02	2.65	3.35	3.87	4.55	5.09	5.93
C. Difco Czapek	.74	1.74	2.79	3.70	4.58	5.40	6.50	7.05	8.10
D. Difco lima bean	.69	1.50	2.00	3.34	3.99	4.72	5.54	6.34	7.29
E. Difco pot.-dext.	.72	1.47	2.24	3.07	3.82	4.49	5.35	6.12	7.39
F. Difco prune	.72	1.42	2.22	2.92	3.62	4.30	5.00	5.69	6.57
G. Sweet potato	.77	1.64	2.60	3.55	4.25	5.25	6.13	6.95	8.22
H. V-8	.80	1.74	2.62	3.80	4.50	5.45	6.48	7.02	8.35
I. Broccoli leaf	.70	1.50	2.60	3.55	4.47	5.30	6.30	7.23	8.28
J. Brussels spr.	.67	1.35	2.47	3.35	4.17	5.07	5.98	6.82	7.50
K. Cabbage leaf	.70	1.53	2.75	3.74	4.69	5.57	6.57	7.48	8.53
L. Chinese cab.	.70	1.54	2.60	3.54	4.49	5.37	6.25	7.32	8.27
M. Collard leaf	.80	1.57	2.70	3.62	4.59	5.45	6.32	7.30	8.28
N. Caulif. leaf	.77	1.57	2.64	3.54	4.47	5.32	6.28	7.27	8.18
O. Kale leaf	.70	1.65	2.70	3.75	4.77	5.55	6.67	7.57	8.57
P. Kohlrabi leaf	.70	1.60	2.67	3.67	4.62	5.47	6.58	7.52	8.48
Q. Mustard leaf	.69	1.39	2.37	3.20	3.95	4.67	5.47	6.25	7.22
R. Radish leaf	.75	1.52	2.57	3.49	4.34	5.09	5.97	6.77	7.88
S. Rape leaf	.65	1.58	2.69	3.60	4.63	5.37	6.47	7.25	7.85
T. Rutabaga leaf	.80	1.64	2.67	3.50	4.42	5.67	6.05	6.80	8.05
U. Turnip leaf	.72	1.44	2.31	3.07	3.92	4.57	5.57	6.29	7.32

*Capital letters in front of the media correspond to those of Figure 25.

Difco corn meal agar proved to be the least desirable as a culture medium for this fungus, although it grew well and in a characteristic manner. Spore production was exceedingly uniform on all media in respect to quantity, quality and appearance. Zonation was very prominent and the concentric rings and sooty black color definitely characterize this fungus in culture and are useful in separating it from other Alternaria spp. on crucifers.

TABLE 16

DESCRIBING GROWTH CHARACTERS OF ALTERNARIA RAPHANI ON VARIOUS HARD AGAR MEDIA AFTER NINE DAYS AT 20°C

Medium	Diameter in cm	Sporulation	Other Characteristics of Colonies
A. Difco bean pod agar pH 5.7	5.67	Several mostly single, chlamydospores abundant	Cottony near center, thin toward margin, whitish to olive gray, moniliform mycelium
B. Difco corn meal agar pH 5.7	6.33	Several single or in chains of 2 rarely 3, chlamydospores abundant	Thin aerial near center, moniliform and submerged mycelium, brownish gray
C. Difco Czapek agar pH 7.3	7.79	Abundant terminal aerial pseudochlamydospores, chlamydospores, aggregated	Cottony, also submerged, moniliform mycelium, whitish to smoky gray
D. Difco lima bean agar pH 7.2	7.09	Many terminal, aerial pseudochlamydospores and abundant chlamydospores	Thin, superficial slightly aerial near center, submerged mycelium moniliform gray
E. Difco potato-destrose agar pH 5.9	7.09	No spores abundant submerged, smaller aggregated chlamydospores	Cottony, whitish brown above, submerged, dark brown to nearly black below
F. Difco prune agar pH 5.7	6.60	Several, single spores, terminal submerged pseudochlamydospores, chlamydospores abundantly, large	Thin, more submerged than superficial, dark brown to olive gray, abundant moniliform mycelium

TABLE 16--Continued

Medium	Diameter in cm	Sporulation	Other Characteristics of Colonies
G. Sweet potato-agar pH 6.3	7.14	Abundant submerged chlamydospores aggregated large, round to irregular shape	Slightly aerial, mostly submerged, abundant moniliform mycelium, smoke color on the top and olive black on bottom
H. V-8 agar pH 7.3	7.75	Abundant, mostly single or 2, chlamydospores abundant, aggregated	Thin, superficial, whitish to brownish gray from the margin toward the center
I. Broccoli leaf agar pH 6.0	4.94	Abundant, singly or in chains of 2 to 6, chlamydospores abundant submerged	Thin margin, cottony toward the center, brown to smoky gray, moniliform mycelium
J. Brussels sprout leaf agar pH 6.3	4.75	Abundant terminal, aerial pseudochlamydospores, and chlamydospores	Slightly superficial and thin growth toward the margin, moniliform mycelium, brown to smoky gray
K. Cabbage leaf agar pH 6.7	5.97	Few spores, aerial pseudochlamydospores, aerial and submerged chlamydospores abundant	Cottony, intertangled with chlamydospores, aerial or submerged, mycelium moniliform, light to smoky gray
L. Chinese cabbage leaf agar pH 6.8	5.60	Abundant in chains of 2-4, terminal pseudochlamydospores both aerial and submerged	Aerial, moniliform mycelium, light brown to gray
M. Collard leaf agar pH 6.5	6.82	Abundant in chains of 2-6, abundant pseudochlamydospores and chlamydospores	Cottony, intertangled with chlamydospores, aerial or submerged, mycelium moniliform, light to smoky gray

TABLE 16--Continued

Medium	Diameter in cm	Sporulation	Other Characteristics of Colonies
N. Caulif. leaf agar pH 6.4	4.62	Abundant single or in chains of 2-5, chlamydospores abundant	Superficial, thin, aerial near the center, moniliform mycelium, light to dark gray
O. Kale leaf agar pH 6.7	6.62	Abundant single or in chains of 2-4 on branched conidiophores, chlamydospores abundant	Cottony near the center and rather thin toward the margin, moniliform mycelium, to smoky gray
P. Kohlrabi leaf agar pH 7.0	6.43	Abundant single or in chains of 2-4 on branched conidiophores, chlamydospores abundant	Cottony, thin oil drops observed, zonate
Q. Mustard leaf agar	5.89	Abundant, single or in chains of 2-5, chlamydospores abundant	Cottony, radiated in the submerged mat, light or gray to smoky or dark gray
R. Radish leaf agar pH 5.9	5.72	Abundant terminal spore like pseudochlamydospores and chlamydospores	Slightly aerial, light to smoky gray, thin margin
S. Rape leaf agar pH 6.3	6.78	Abundant, terminal pseudochlamydospores, chlamydospores	Aerial near the center and thin toward the margin, gray on the top, smoky gray to dark gray below
T. Rutabaga leaf agar pH 6.2	6.85	Abundant terminal spore like pseudospores, chlamydospores	Aerial or submerged, zonate, gray to dark gray

TABLE 16--Continued

Medium	Diameter in cm	Sporulation	Other Characteristics of Colonies
U. Turnip leaf agar pH 6.8	5.42	Abundant pseudochlamydospores, chlamydospores	Thin, cottony toward the center, small to large brown oil drops, light to smoky or dark gray

The growth characters of A. raphani on the various media were generally uniform. The colonies were somewhat superficial but definitely submerged in several instances. The growth was typical throughout and readily distinguished from other Alternaria spp. parasitic on crucifers. An abundance of pseudochlamydospores and chlamydospores produced was individualistic with it.

TABLE 17

DIAMETER OF COLONIES OF ALTERNARIA RAPHANI IN CM GROWN AT 20°C FOR NINE DAYS ON VARIOUS HARD AGAR MEDIA

Agar Medium*	Diameter of Colonies on Indicated Days								
	1	2	3	4	5	6	7	8	9
A. Difco bean pod	.50	.97	1.98	2.50	3.14	3.79	4.54	4.92	5.67
B. Difco corn meal	.50	.97	1.84	2.40	2.67	3.30	4.17	5.60	6.33
C. Difco Czapek	.60	1.65	2.57	3.72	4.60	5.33	6.28	6.80	7.79
D. Difco lima bean	.67	1.58	2.65	3.50	4.14	4.88	5.67	6.40	7.09
E. Difco pot.-dext.	.60	1.67	2.49	3.24	3.70	4.82	5.35	6.02	7.09
F. Difco prune	.60	1.55	2.04	2.92	3.54	4.37	5.15	5.95	6.60
G. Sweet potato	.57	1.07	2.29	3.19	3.66	4.25	5.50	6.22	7.14
H. V-8	.60	1.20	2.54	3.57	4.20	5.15	6.20	7.08	7.75
I. Broccoli leaf	.68	1.37	2.08	2.54	2.92	3.43	4.07	4.49	4.94
J. Brussels spr.	.53	1.45	2.18	2.65	2.92	3.54	4.12	4.50	4.75
K. Cabbage leaf	.70	1.45	2.53	3.33	3.95	4.40	5.27	5.55	5.97
L. Chinese cab.	.63	1.29	2.18	2.75	3.30	3.97	4.64	5.02	5.60
M. Collard leaf	.65	1.48	2.30	2.96	3.73	4.33	5.23	6.19	6.82
N. Cauilf. leaf	.52	1.15	2.34	2.52	2.88	3.47	3.88	4.20	4.62
O. Kale leaf	.60	1.47	2.13	2.62	3.22	3.60	4.42	5.40	6.62
P. Kohlrabi leaf	.75	1.70	2.57	3.39	3.87	4.57	5.22	5.99	6.43
Q. Mustard leaf	.62	1.33	2.05	2.70	3.33	3.84	4.55	5.18	5.89
R. Radish leaf	.55	1.32	2.15	2.79	3.05	3.90	4.65	5.20	5.72
S. Rape leaf	.62	1.52	2.45	3.17	3.89	4.54	5.20	6.02	6.78
T. Rutabaga leaf	.70	1.53	2.22	2.93	3.67	4.18	5.00	5.88	6.85
U. Turnip leaf	.53	1.10	1.95	2.52	3.03	3.50	4.22	4.54	5.42

*Capital letters in front of the media correspond to those of Figure 25.

The growth of A. raphani on the various media was relatively uniform. It is worthy to note that the radish leaf decoction medium was apparently less satisfactory to the parasite than highly synthetic media. Generally, however, the response of the fungus was similar in all cultures, the variations were slight and insignificant. It is notable, however, that in every medium there was produced aggregations of pseudochlamydospores and well-formed chlamydospores not found in cultures of the other Alternaria spp.

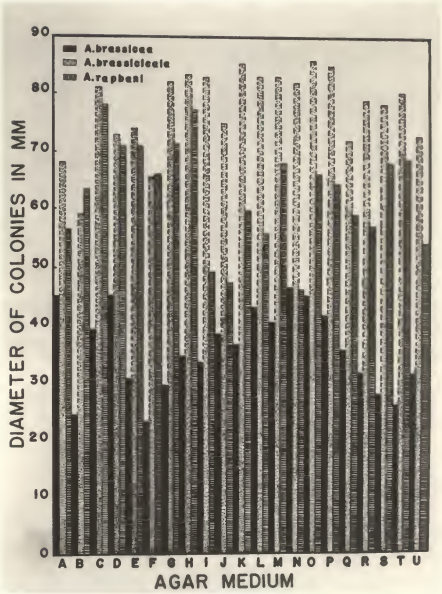

Fig. 25.--Diameter of colonies of Alternaria brassicae, A. brassicicola and A. raphani grown at 24°C for nine days on the various agar media indicated alphabetically from A to U corresponding to the letters used in Tables 13, 15 and 17.

by all three of these fungi. The so-called cabbage group of medium leaf preparations were more favorable for the growth and sporulation of A. brassicicola. Alternaria raphani also grew very well on these media. On the other hand, A. brassicicola grew best and produced abundant spores on a wide range of media other than those prepared from foliage of the cabbage group. The extent of growth in mm as measured over the nine-day period for the three Alternaria spp. is graphically shown in Figure 25, which shows the types of media used, indicated by the symbols at the bottom of the graph and the colony diameter at the left margin in mm. Alternaria brassicicola produced the most extensive colonies on all but Difco corn meal medium, in which case A. raphani grew farther. Alternaria brassicae was slower growing, produced the least extensive colonies and was least favored by any of the media, with the possible exception of the cauliflower leaf agar media. The extensive sporulation, black sooty color, and zonations characterize A. brassicicola. The pseudochlamydospores and chlamydospore production apply to A. raphani and the brown color of the colony and spores identify A. brassicae distinctively.

Growth on Leaf Decoctions

A series of broccoli leaf decoctions containing 2 per cent agar was prepared by boiling 200 g of chopped broccoli leaves for thirty minutes in 500 ml of water, as mentioned by Riker and Riker (61). The water lost from boiling was restored. Two hundred fifty or half of the 500 ml extract was diluted with an equal part of distilled water totaling a diluted solution of 500 ml. Half of this first dilution was again diluted with an equal part of distilled water. This process was repeated five

times and 250 ml of distilled water containing 10 g agar was added to each of the 250 ml remaining extracts, resulting in a series of the leaf decoction agar dilutions containing 6.25, 12.5, 25, 50, 100, 200 and 400 g per liter of the original extract of the 200 g of chopped broccoli leaves. The media were sterilized. The initial pH values determined from random selected tubes of each of the seven dilutions were 5.6, 5.7, 5.6, 5.7, 5.7, 5.6, and 5.3, respectively, from the most dilute to the highest concentrations. At the end of eight days the initial pH readings had changed to 7.2, 7.2, 7.3, 7.5, 7.6, 7.3, and 7.2, respectively. Each plate was planted with a 3 mm disc of agar bearing the fungus as inoculum. Three replications were made. They were incubated at 24°C and the rate of growth was measured daily by determining the diameter of each colony. A hand lens was sometimes used to determine the margin of very thin growth. The growth characters as tabulated in Table 18 are shown in graphic form (Fig. 26).

The results indicate that each of the three Alternaria spp. grew well and sporulated on all of the concentrations of the leaf decoction. The average variation in colony size of A. brassicae growing on all concentrations was less than 1/2 cm after eight days. As a matter of fact, the greatest concentration produced the least extensive growth. The growth was very thin at the weaker concentrations and became thicker and more dense as the concentration increased. It was also true that the greater amount of spore formation occurred progressively from the thin to the thick colonies, or from the weakest concentrations to the greater concentration. The average variation in colony size of A. brassicicola was less than 1 cm from the smallest to the most extended growth. In

TABLE 18

DIAMETER OF COLONIES OF ALTERNARIA SPP. IN CM GROWN AT 24°C FOR EIGHT DAYS ON INDICATED CONCENTRATIONS OF BROCCOLI LEAF DECOCTION AGAR MEDIA

Pathogen	Days	Diameter of Colonies Grams of Leaf Decoction per Liter						
		6.25	12.5	25	50	100	200	400
Alternaria brassicae	1	.55	.60	.62	.64	.69	.60	.65
	2	1.77	1.35	1.30	1.23	1.32	1.17	1.22
	3	1.95	2.03	1.92	1.97	2.00	1.87	1.80
	4	2.50	2.55	2.43	2.50	2.54	2.40	2.30
	5	3.09	3.05	3.02	3.14	3.17	3.00	2.88
	6	3.72	3.58	3.83	3.74	3.80	3.64	3.51
	7	4.37	4.45	4.58	4.39	4.50	4.29	4.26
	8	5.00	5.05	5.14	4.90	5.05	4.90	4.87
Alternaria brassicicola	1	1.07	1.02	.90	.99	1.05	1.03	.99
	2	2.07	1.98	1.88	1.94	2.04	2.04	2.01
	3	3.09	2.95	2.81	2.96	3.03	3.13	3.07
	4	3.97	3.76	3.69	3.80	3.90	4.03	3.99
	5	4.86	4.72	4.62	4.70	4.82	5.00	4.99
	6	5.72	5.63	5.58	5.72	5.90	6.02	6.03
	7	6.74	6.52	6.73	6.62	6.89	7.05	7.17
	8	7.60	7.39	7.47	7.55	7.80	8.05	8.14
Alternaria raphani	1	.90	.97	.90	.90	.82	.92	.97
	2	1.87	1.93	1.80	1.73	1.54	1.85	1.95
	3	2.84	2.88	2.57	2.57	2.16	2.63	2.80
	4	3.47	3.40	3.27	3.24	2.87	3.23	3.27
	5	4.35	4.07	4.05	3.93	3.35	3.57	3.44
	6	5.25	4.82	4.87	4.73	3.95	3.98	3.73
	7	6.42	5.93	5.70	5.80	4.65	4.54	4.65
	8	7.25	6.79	6.47	6.77	5.55	5.14	5.27

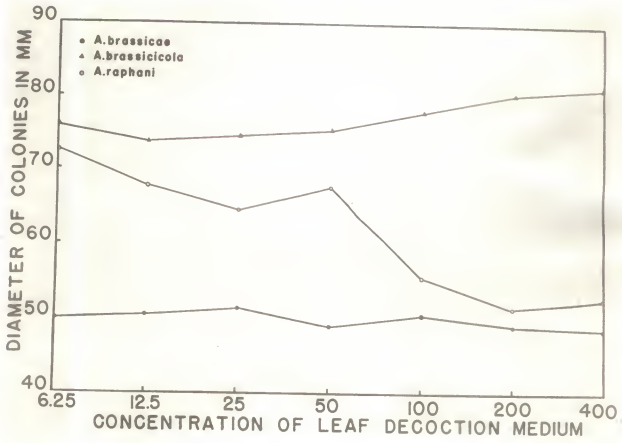

Fig. 26.--Diameter of colonies of Alternaria brassicae, A. brassicicola and A. raphani grown at 24°C for eight days on the various concentrations of broccoli leaf decoction media.

this respect the measurements were narrowly variable and resembled the similar results produced by A. brassicae. The colonies were thinner on the most dilute solutions and became thicker gradually as the concentrations increased. In this series of cultures the more concentrated solutions not only produced the thicker colonies but also supported the most extensive growth. The average variation in colony size of A. raphani was less than 2 cm which was very pronounced not only because of the wide variation but also because the most extensive developed colonies were in the most dilute concentrations. The more concentrations supported the denser colonies but those colonies appeared to be somewhat restricted in extent. Sporulation gradually increased as the concentrations increased and in this instance showed the greatest production of chlamydospores and conidia on the denser smaller colonies. In comparison A. brassicicola growth on all of the concentrations was more extensive in every concentration than was produced by A. brassicae and A. raphani on any concentration. These data show that the rate of growth was distinctive in each of these fungi. It is also shown that the higher concentrations produced the denser colonies and heavier spore formation in each of these fungi.

Effect of Temperature

The effect of temperature on the growth and development of Alternaria spp. pathogenic to crucifers has been of considerable interest to various writers. Weimer (83) reported a slow decay of cauliflower caused by A. brassicae at 0 to 9°C and at 20° to 25°C. Rangel (60) found that the optimum temperature for spore germination was between 17.2° and 21.1°C. The germination was sensitive to the variation of temperatures.

Neergaard (51) observed the optimum temperature for mycelial growth was about 23°C with a sharp drop at higher temperatures, as shown in his graph. The optimum temperature for growth of A. raphani as reported by Neergaard was the same as for A. brassicae; however, A. raphani showed a slightly wider optimal range, 22°C to 26°C, as reported by Atkinson (3). He also pointed out that this pathogen grew rapidly in potato-dextrose agar and was highly sensitive to temperature changes. In A. brassicicola the temperature most favorable for infection of cabbage and cauliflower heads was reported by Walmer (82) to be between 25° to 31°C. Ramsey, et al. (59) stated that the temperatures of about 2° to 7.5°C, accompanied by low relative humidity greatly inhibited the development of decay. The range of temperature for spore germination reported by Walker (77) was between 1° to 40°C, with the optimum temperature between 33° to 35°C. There was no sharp optimum temperature for spore germination recorded by Rangel (60). The optimum temperature for growth of mycelium was close to 25°C, and it dropped sharply at 30°C (51, 82).

The following experiment was conducted in order to compare the responses of growth to various temperatures of A. brassicae, A. brassicicola and A. raphani. The 3-millimeter discs of the pathogens cut with a cork borer from the edge of colonies grown in V-8 agar for five days were placed at the center of Petri dishes containing 15 ml of potato-dextrose agar that showed a reaction of pH 5.6. The final reaction taken from the small pieces of medium of different plates was pH 5.8. Three replications of each pathogen were incubated at temperatures ranging from 4° to 36°C with 4°C increments. The rate of growth was determined by calculating the average diameter of colonies measured at right

angles to each other every day for seven days. The experiment was terminated when the mycelium at optimum temperatures reached the edge of the Petri dishes. The results of growth of these pathogens are tabulated in Table 19 and are also given in graphic form (Fig. 27).

The optimum temperature for growth of A. brassicae was between 20° and 24°C on potato-dextrose agar (Fig. 28, 29). The extent of growth at lower temperatures was less and less on a gradual scale down to 4°C where the fungus survived but did not expand. At temperatures above the optimum range the expansion of the colonies was prevented and very little development occurred above 30°C. The colonies were thin at the temperature below optimum and comparatively thicker above the optimum. Sporulation was scanty below 16°C and above 28°C with the more abundant production at the optimum temperatures.

Alternaria brassicicola produced some growth at the extreme temperatures of 4°C and 36°C to which it was exposed with an optimum between 24° and 28°C (Fig. 29, 30). At the end of seven days the colonies growing at optimum temperatures extended nearly to the edges of the Petri dishes. The expansion of the colonies was gradual at temperatures lower than 28°C whereas at 32° there was an abrupt limitation on the expansion of the colony. This inhibition occurred at 36°C but was not too different than at the 32°C temperature. These colonies were thin at the extreme temperatures but in all instances were dark colored. Between 16°C and 32°C their surfaces were a sooty black color produced by the production of conidia.

The extent and limitation of A. raphani expansion of colonies grown on potato-dextrose agar at various temperatures is shown graphically

TABLE 19

DIAMETER OF COLONIES OF ALTERNARIA SPP. IN CM GROWN IN 2 PER CENT POTATO-DEXTROSE AGAR FOR SEVEN DAYS AT INDICATED TEMPERATURES

Pathogen	Days	Diameter of Colonies in Different Temperatures (°C)								
		4	8	12	16	20	24	28	32	36
Alternaria brassicae	1	0	a	a	.47	.66	.69	.45	0	0
	2	0	.50	.50	.71	1.05	1.15	.70	a	0
	3	.45	.55	.75	.95	1.90	1.80	1.05	a	0
	4	.51	.60	.92	1.25	2.67	2.50	1.25	a	0
	5	.62	.65	1.10	1.53	3.35	3.25	1.55	a	0
	6	.75	.95	1.35	1.90	4.00	4.20	2.20	a	0
	7	.85	1.05	1.65	2.25	4.70	5.30	2.65	a	0
Alternaria brassicicola	1	a	a	a	.64	.70	.88	.72	.48	.45
	2	.45	.50	.61	1.02	1.75	2.00	1.95	.75	.70
	3	.57	.57	.90	1.85	2.80	2.85	2.95	1.00	1.00
	4	.71	.65	1.51	2.60	3.82	4.04	4.05	1.16	1.20
	5	.84	.80	1.90	3.30	4.90	4.95	4.99	1.55	1.50
	6	1.00	1.55	2.35	3.90	6.05	6.10	6.10	1.95	1.90
	7	1.30	1.75	2.80	4.65	7.00	7.21	7.25	2.51	2.28
Alternaria raphani	1	.40	.40	.43	.71	.82	1.09	1.14	.45	0
	2	.45	.50	.65	1.25	1.80	2.25	2.35	.70	0
	3	.60	.65	1.25	1.95	3.05	3.43	3.60	.74	0
	4	.77	.81	1.80	2.77	4.15	4.77	5.24	.77	a
	5	.93	.99	2.25	3.40	5.21	5.95	6.05	.83	a
	6	1.24	1.28	2.65	4.10	6.44	7.24	7.62	.85	a
	7	1.55	1.59	3.15	4.76	7.50	8.49	8.59	.90	a

a: Trace, no measurement.

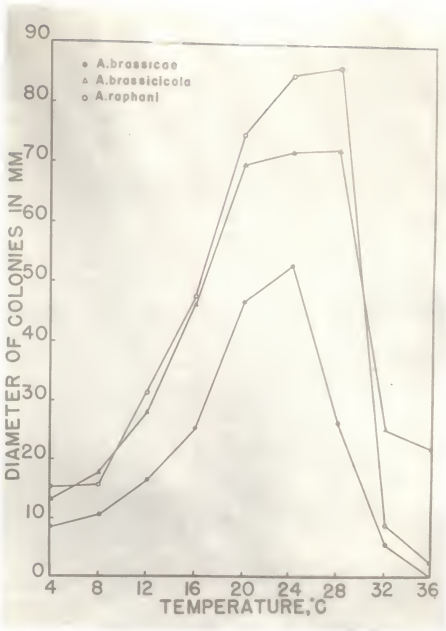

Fig. 27.--Diameter of colonies of Alternaria brassicae, A. brassicicola and A. raphani after seven days in 2 per cent potato-dextrose agar, incubated at indicated temperatures.

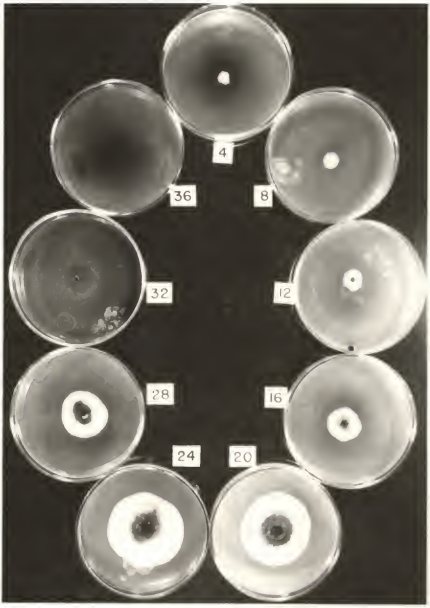

Fig. 28.--Colonies of Alternaria brassicae after seven days on 2 per cent potato-dextrose agar, incubated at indicated centigrade temperatures.

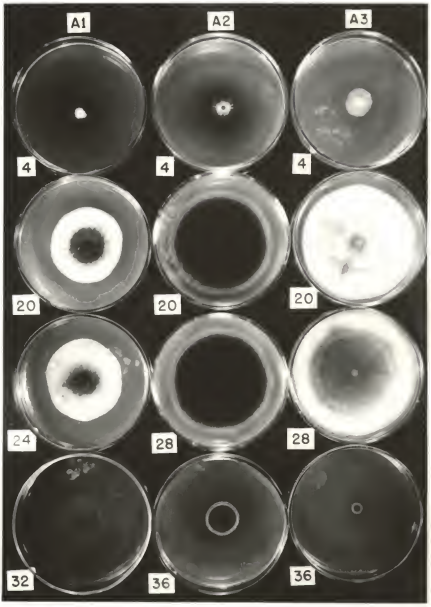

Fig. 29.--Colonies of Alternaria brassicae (A1), A. brassicicola (A2), and A. raphani (A3) grown in potato-dextrose agar for seven days at indicated centigrade temperatures.

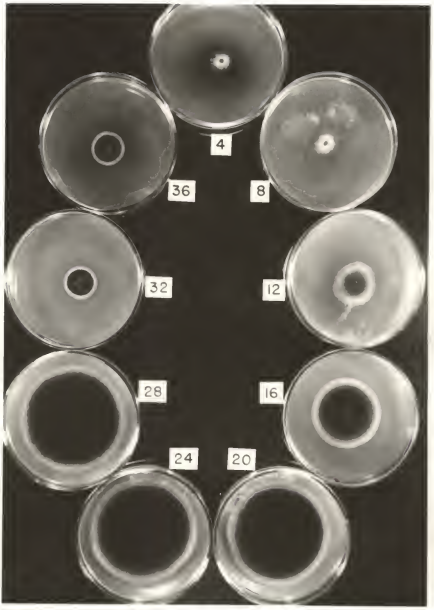

Fig. 30.--Colonies of Alternaria brassicicola after seven days on 2 per cent potato-dextrose agar, incubated at indicated centigrade temperatures.

(Fig. 27). The fungus produced some growth in a gradual decline down to 4°C from the optimum temperature indicated at between 24° and 28°C (Fig. 29, 31). At the temperatures above the optimum growth ceased at 32°C and thereafter. The mycelium was light greenish gray in color and only moderately dense. Some zonation developed but sporulation was scanty even at the optimum ranges of temperature. Chlamydospores and pseudochlamydospores were produced at all but the lower of 8°C. The graph showing comparative temperature ranges indicates that A. brassicicola grew at a wider range of temperatures than either of the other Alternaria spp. It also shows that rate of expansion of A. raphani was more extensive than the others and that it was more effectively inhibited by slight changes of temperatures than the others. Alternaria brassicae was slower growing at about the same cardinal temperatures (Fig. 28). These fungi appear individually distinctive in culture at the optimum temperatures in relation to color, zonation and spore production. The growth of each of these fungi was more sensitive above the optimum temperatures than below it.

Effect of Hydrogen Ion Concentration

The effect of hydrogen ion concentration on growth of Alternaria brassicae, A. brassicicola and A. raphani was studied by using 3 per cent potato-dextrose agar as a medium. About 250 ml of the media in each 500 ml flask after sterilizing in an autoclave were adjusted to a series of pH values by using 1/N HCl and 1/N NaOH. The initial pH values were 2.0, 2.9, 3.9, 5.0, 6.0, 7.1, 8.0, 9.1 and the final pH values determined after nine days from pieces of the medium collected in different plates of each series were 2.1, 2.75, 3.8, 5.2, 6.3, 6.9, 7.5, and 8.0 respectively. A

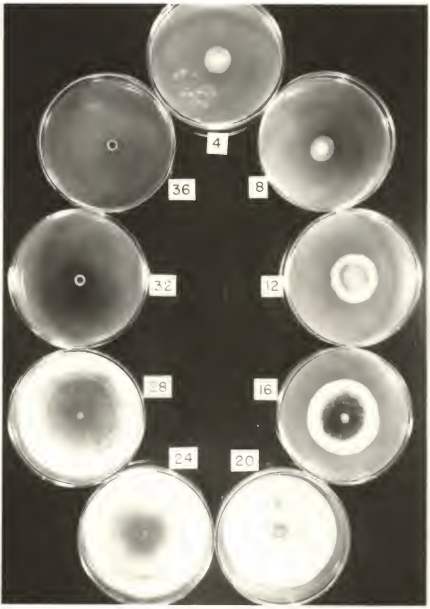

Fig. 31.--Colonies of Alternaria raphani after seven days on 2 per cent potato-dextrose agar, incubated at indicated centigrade temperatures.

3-millimeter disc containing the pathogens grown on V-8 agar for five days was placed in each Petri dish in each series. Three replications were used and incubated at 24°C. The rate of growth during a nine day period was measured daily by taking two readings at right angles to each other.

The data acquired from the growth of all three pathogens are tabulated in Table 20. The results of their growth are also shown in the physical appearances (Fig. 32, 33, 34) and in the graphic form (Fig. 35).

The optimum growth of A. brassicae developed on the medium that showed a reading of pH 8; however, the growth at pH 7.1 was very nearly the same so that the exact pH reading should be closer to 7.1 than 9.1 The calculated optimum should be about pH 7.8. The fungus barely survived at the concentrated acid end of the pH scale but showed considerable growth at pH 5 and thereafter to the end of the alkaline range through pH 9. The colonies were grayish to light brown in color and spores were produced at all concentrations above pH 5. A faint zonation became conspicuous close to neutrality on both acid and alkaline media.

A. brassicicola grew very well in pH concentrations of pH 5 and above. The optimum development was at pH 7.1 although almost equal production was to be observed on both sides of neutrality. The alkaline range produced exceptionally vigorous growth whereas very thin colonies appeared below pH 5 with no growth below pH 3. Heavy sporulation was found in all the concentrations above pH 5. The colonies were a conspicuous sooty black resulting from the dense production of spores in long chains, with a narrow white advancing margin.

The most extensive growth of A. raphani was on the alkaline side of neutrality although there was no indication of poor development on an

TABLE 20

DIAMETER OF COLONIES OF ALTERNARIA SPP. IN CM GROWN IN 2 PER CENT POTATO-DEXTROSE AGAR AT 20°C FOR NINE DAYS AT THE INDICATED HYDROGEN ION CONCENTRATIONS

Pathogen	Days	Diameter of Colonies at Different pH							
		2.0	2.9	3.9	5.0	6.0	7.1	8.0	9.1
Alternaria brassicae	1			a	a	a	a	a	a
	2			.48	.64	.58	.50	.50	.45
	3			.70	1.05	1.10	.80	.80	.60
	4			.75	1.60	1.85	1.70	2.00	1.28
	5			.87	2.12	2.40	2.25	2.80	1.76
	6			.95	2.81	3.00	3.20	3.66	2.48
	7			1.15	3.43	3.45	4.08	4.57	3.65
	8			1.39	4.27	4.65	5.03	5.47	4.62
	9			1.47	4.60	5.07	5.93	6.04	5.06
Alternaria brassicicola	1			.48	.60	.70	.85	.95	.58
	2			.73	1.32	1.52	1.75	1.75	.95
	3			1.25	2.23	2.52	2.58	2.56	1.63
	4			1.50	3.05	3.28	3.40	2.98	2.54
	5			1.76	3.75	4.05	4.28	3.85	3.04
	6			2.09	4.71	5.05	5.23	4.91	4.00
	7			2.40	5.63	6.05	6.47	5 R6	4.82
	8			2.80	6.47	6.85	7.22	7.00	5.74
	9			3.10	7.46	7.80	8.30	7.98	6.62
Alternaria raphani	1			.48	.65	.95	.95	.85	.67
	2			.70	1.35	1.80	1.78	1.55	1.25
	3			1.05	2.25	2.75	2.73	2.55	2.22
	4			1.15	2.99	3.55	3.60	3.46	3.12
	5			1.38	3.80	4.52	4.53	4.40	4.37
	6			1.67	4.83	5.58	5.65	5.50	5.10
	7			1.97	5.82	6.65	6.70	6.56	5.90
	8			2.25	6.85	7.73	7.73	7.75	7.16
	9			2.48	7.75	8.65	8.87	8.81	8.26

a: Trace and aerial growth, no measurement.

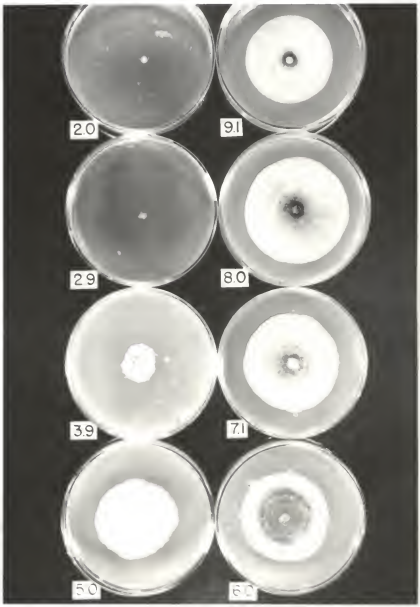

Fig. 32.--Alternaria brassicae grown on potato-dextrose agar at 20°C for nine days at the indicated hydrogen ion concentrations.

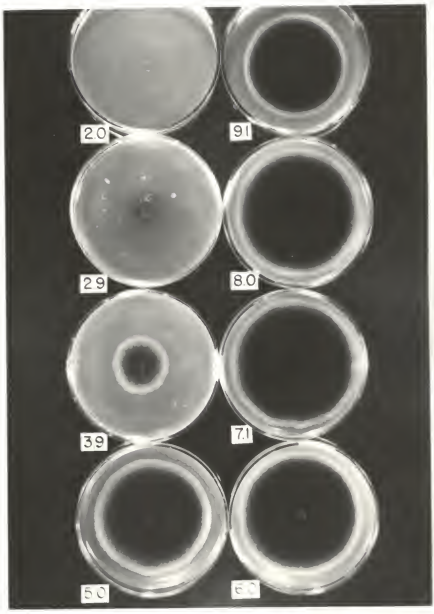

Fig. 33.--Alternaria brassicicola grown on potato-dextrose agar at 20°C for nine days at the indicated hydrogen ion concentrations.

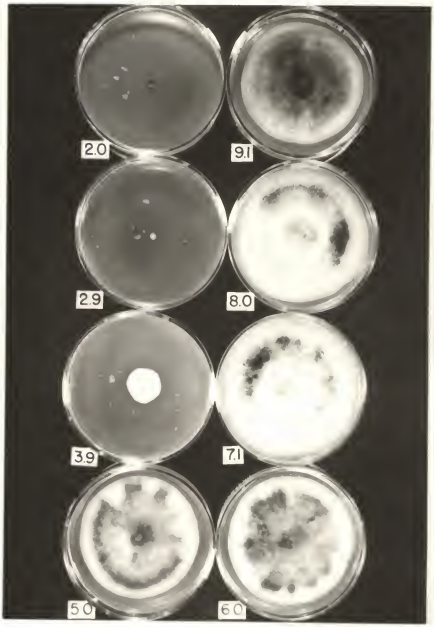

Fig. 34.--Alternaria raphani grown on potato-dextrose agar at 20°C for nine days at the indicated hydrogen ion concentrations.

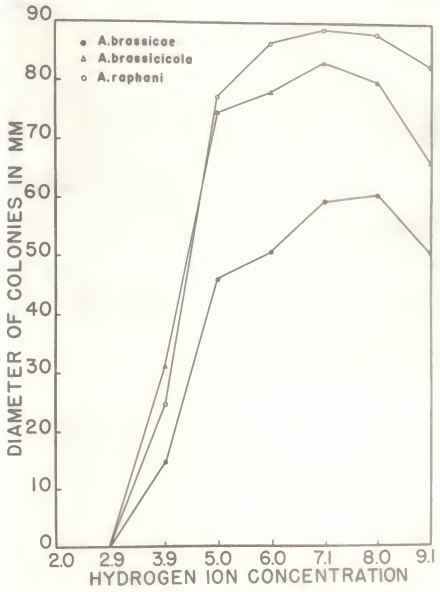

Fig. 35.--Diameter of colonies of Alternaria brassicae, A. brassicicola and A. raphani grown on potato-dextrose agar at 24°C for nine days at the indicated hydrogen ion concentrations.

acid media except below pH 5. The growth was poor below pH 4 with none on the more acid media. The colonies were whitish to light gray in appearance on the upper surface of the medium and rather black on the lower surface. Conidia production was scanty in all cultures and chlamydospores were present in all cultures above pH 5.

The minimum, optimum and maximum development of these fungi in relation to the pH reaction of the growth medium is shown (Fig. 36). All of these fungi grew better on the alkaline side of neutrality and at pH 9.1 was the earliest indication of an inhibiting affect of the medium. Alternaria raphani developed the most extensive growth over a greater pH range than the others, and A. brassicae responded to the medium in the poorest manner comparatively. Alternaria brassicicola produced the greatest number of conidia of these three fungi and A. raphani the fewest number of conidia although the chlamydospores were more plentiful here than in the other lower pH cultures. The curves in the graph (Fig. 35) indicate that A. raphani and A. brassicicola are almost equally favored by the media while A. brassicae appears to be considerably restrained by the media.

Effect of Light

Marsh et al. (44) compiled several reports dealing with the effect of light on fungi. It appears that light might effect reproduction, morphology, pigmentation, and phototropic phenomena. They observed that sporulation of Alternaria, Fusarium, and Diplodia were most frequently influenced by light. Light may stimulate or inhibit sporulation. Bjorusson (7) found that a yellow mat of Stemphylium mycelium without spores was produced in continuous darkness and a black mat of spores with

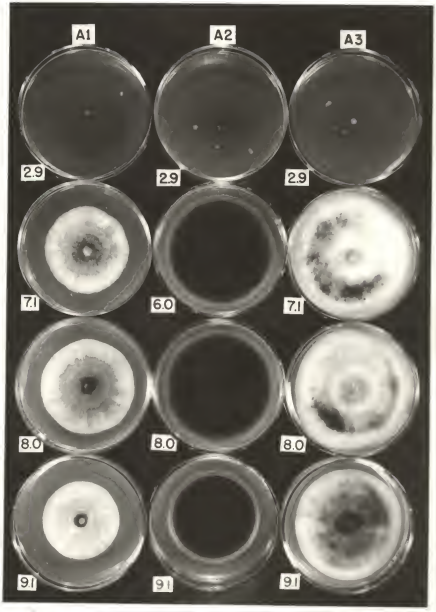

Fig. 36.--Colonies of Alternaria brassicae (A1), A. brassicicola (A2), and A. raphani (A3) grown on potato-dextrose agar for nine days at indicated hydrogen ion concentrations.

a slight growth of white mycelium was produced in continuous light. It also occurred with Stemphylium floridanum as reported by Hannon and Weber (30). They pointed out that cultures growing at room temperature with normal cycles of day and night produced conidia in conspicuous zones whereas other cultures in continuous light showed abundant sporulation without zonation. Weston (85) said that sporulation of Alternaria solani was induced by a high intensity of visible white light and continued high light intensities increased pigmentation. Klaus (39) claimed that only weak light was enough to induce sporulation, but complete darkness inhibited it. Johnson and Halpin (36) found that typical muriform conidia was consistently obtained in five-week old cultures incubated in continuous darkness and under increasing light intensities the conidia produced were elongated and narrow. Light was also reported by Witsch and Wagner (87) as a necessity for the formation of sterigmata of Alternaria dauci. In constant fluorescent light the hyphae were clearly septate, thick-walled and with many sterigmata but not conidia. In constant darkness hyphae were thin walled, and few conidia formed in the area of the inoculum. Gallemaerts (25) found zonation produced by Alternaria tenuis subjected to daily changes of light and darkness, but in continuous light or in continuous darkness no zonation occurred. Isaac and Abraham (34) postulated that the zonation of Verticillium laterituim was due to the stimulation of sporulation effected by the specific intensity, period, and wavelength of light, followed by at least a nine-hour period of darkness. They concluded that zonation might be due to the reduction of sporulation resulting from poor development of mycelium effected by temperature, or

probably due to an excessive condensation causing a physical collapse of some of the conidiophores.

To investigate the effects of light and darkness on zonation formation of Alternaria brassicicola, the following trials were conducted. Discs of fungus-invaded agar, 3 mm in diameter were transferred to the centers of Petri dishes containing 15 ml potato-dextrose agar. One set was wrapped with black paper and kept in a cabinet, the second was exposed to fluorescent light of about twenty foot-candales. Both were kept at 20°C. The third set was exposed to day and night influences of light in the laboratory. After four days the light-exposed plates were subjected to darkness for 0, 2, 4, 6, 8, 10 and 12 hours, and the darkness-exposed plates were exposed to light for 0, 1/2, 1, 3, 6, 15, 30, 60 and 120 minutes, and both were taken back to their original location. Two replications of each were used at random. The observations were taken six days after treatment (Fig. 37). The number of spores per 3 mm disc in water plus a few drops of ethyl alcohol was determined by a Hemocytometer.

The results showed that the cultures which were exposed to normal day and night showed alternate zones which were darkest beginning from the inner rings and lighter toward the outer rings, with a narrow lighter area between rings. The darker color was due to the heavier spore formation. The number of spores per 3 mm disc averaged from five discs of the first, second, third, and fourth zones next to the central disc were 24,500; 19,000; 13,000 and 7,500 spores respectively. The average number of spores per disc from ten discs radiated from the original disc kept in the continuous light was 15,500 spores. The colonies looked slightly aerial and were of a dark color. The average number of spores in the

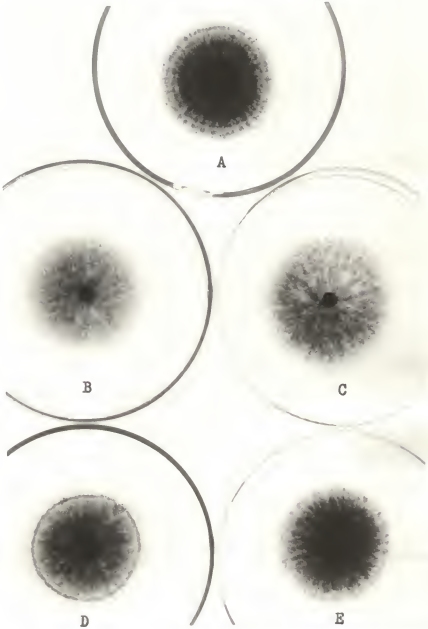

Fig. 37.--Colony of Alternaria brassicicola grown on potato-dextrose agar for six days at 20°C. A, alternate day and night; B, continuous darkness; C, continuous light; D, colony kept in darkness except for a thirty minute light exposure; E, colony kept in light except for a twelve hour dark exposure.

plates exposed to continuous darkness was 19,000 spores. The colonies looked smooth, superficial and sooty black. No zonation was observed on those plates which were kept in either continuous light or continuous darkness. Zonation was formed on plates which were changed from light to darkness or vice versa. At least three minutes was required to produce a zonation on plates changed from darkness to light and six hours for those changed from light to darkness. Longer period of change produced sharper zonation. In plates exposed to either continuous light or darkness the colonies appeared darker than the checks. It was also observed that the spores of this fungus produced in continuous darkness were smaller and the cell walls thinner than those produced under continuous light. More spores were produced in continuous darkness than in continuous light.

Pathogenicity

The severity of the diseases of crucifers produced by Alternaria spp. has been reported frequently on different stages of growth of cruciferous plants. Inoculations have been made on certain crucifers by many investigators. Rangel (60) inoculated seeds of cabbage with A. brassicicola before planting and reported a 100 per cent killing of seedlings by pre- and post-emergence. Neergaard (51, 52) concluded his inoculation studies using seedlings of cabbage, wallflower, radish, stock, and candytuft made in test tubes in the laboratory that those seedlings were attacked strongly by A. brassicicola and weakly by A. brassicae. He also reported positive results on cabbage, radish, wallflower, and stock seedlings inoculated with A. raphani. In addition, he made a statement in 1958 that during the last five years both A. brassicae and A. brassicicola were

seed-borne parasites which had been subjected to seed testing for cruciferous seed at Copenhagen, Denmark. Atkinson (3) pointed out that unless radish seeds were severely infected with A. raphani most of the seedlings survived. The inoculation of seeds of radish, stock, and wallflower grown in test tubes showed up to 100 per cent infection for the wild strains and 30 - 80 per cent for the variants. He also stated that increased soil moisture was associated with increased seedling disease. The experiments described below were conducted to compare the susceptibility of the common vegetables in the cruciferae family to each of the three pathogens.

A. Soil inoculated with Alternaria spp.

Fumigated soil was sterilized again by steaming in an autoclave for 2 hours at 15 pound pressure and was divided into four parts. Each part was thoroughly mixed with five Petri dishes of pure cultures of each of A. brassicae, A. brassicicola and A. raphani grown in potato-dextrose agar which were cut into small pieces. One part was left as check. Thirty-nine Petri dishes were filled with each of these contaminated lots of soils and duplicated with the check soil. Twenty-five surface disinfected seeds of each variety of crucifers were planted and covered with a thin layer of those soils. Three replications were made for each crop variety. The covers were kept on the dishes until the seeds germinated. Sterile water was applied frequently by a hand sprayer to prevent drying. The data were collected 6 days after inoculation by counting the number of seedlings which were healthy or infected after they emerged from the soil. The severity of pre- and post-emergence damping-off on different varieties caused by the pathogens is shown in Table 21 and in physical appearances (Fig. 38), supplemented with observational details listed below.

TABLE 21

PERCENTAGE OF PRE- AND POST-EMERGENCE INFECTION OF SEEDLINGS IN SOIL INOCULATED WITH ALTERNARIA SPP

Host	Germination of check	Percentage Infection								
		A. brassicae			A. brassicicola			A. raphani		
		a	b	c	a	b	c	a	b	c
Broccoli	89	2	0	98	0	40	60	0	39	61
Brussels sprouts	52	3	15	82	0	33	67	0	0	100
Cabbage	32	6	1	93	0	87	13	0	46	54
Chinese cabbage	76	2	23	75	0	89	11	0	63	37
Collards	79	2	34	64	0	72	28	0	66	34
Cauliflower	59	0	20	80	0	80	20	0	52	48
Kale	36	0	10	90	0	5	95	0	0	100
Kohlraby	95	8	0	92	0	28	72	0	41	59
Mustard	20	5	9	86	0	35	65	0	0	100
Radish	97	3	3	94	0	2	98	0	6	94
Rape	87	1	2	97	2	2	86	3	0	97
Rutabaga	1	0	0	100	0	100	0	0	0	100
Turnip	84	0	3	97	0	34	66	0	38	62

Percentage infection calculated from the percentage of germination.
a: Healthy plants.
b: Pre-emergence infection.
c: Post-emergence infection.

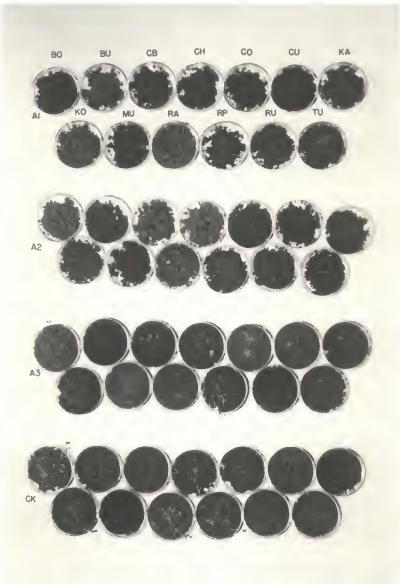

Fig. 38.--A representative replication of Petri dishes containing thirteen varieties of cruciferous seeds on soil inoculated with Alternaria brassicae (A1), A. brassicicola (A2), and A. raphani (A3). CK is a check. The capital letters on the top indicate cruciferous varieties as mentioned in Table 2.

1. Seedlings grown in the check soil. The percentage of germination of radish, kohlrabi, broccoli, rape, turnip, collards, and Chinese cabbage was from 76 - 97 per cent; moderate germination ranging from 32-59 per cent included cauliflower, brussels sprouts, kale and cabbage; there was a low germination of 20 per cent of mustard and only 1 per cent germination of rutabaga. All seedlings were disease free. Some seedlings developed poorly. Where no germination occurred, the seed remained bright colored.

2. Seedlings grown in soil mixed with cultures of A. brassicae. Most of the seedlings were killed after emerging from the soil prior to the spreading of their cotyledons. The infection showed brown, water-soaked lesions beginning from the base of plants near the soil line. The plants fell over followed by the fungus invasion of the hypocotyl and cotyledons (Fig. 39). Many seedlings of brussels sprout, kohlrabi, radish, and rape were killed after fully spreading their cotyledons. Brown, constricted lesions appeared at the base of the plants. Few spots were also found on hypocotyl and cotyledons. By aid of a steroscopic binocular microscope, young light-brown spores were observed over the lesions.

3. Seedling grown in soil mixed with cultures of A. brassicicola. Most seedlings in this lot showed a high percentage of pre-emergence damping-off. Seeds which did not germinate were grayish black and fully covered with spores and mycelium. Post-emergence infection was severe and seedlings were killed before the cotyledons were spread. Small dark-brown to black, water-soaked or sunken spots were observed on those infected plants with badly constricted lesions on the stems at the soil line (Fig. 39, 40).

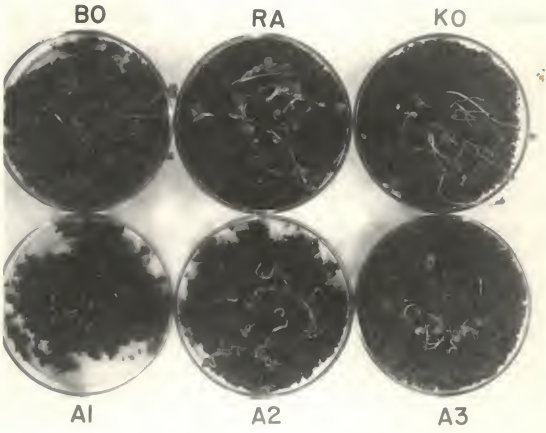

Fig. 39.--Six-day old seedlings of broccoli (BO), radish (RA) and kohlrabi (KO). On the top row the soil was noninoculated and in the bottom one the soil was inoculated with Alternaria brassicae (AI), A. brassicicola (A2) and A. raphani (A3) before planting.

Fig. 40.--Six-day old kohlrabi seedlings removed from the check and Alternaria brassicicola inoculated soils.

4. Seedlings grown in soil mixed with cultures of A. raphani. High percentage of pre-emergence infection occurred with broccoli, cabbage, Chinese cabbage, collard, cauliflower, kohlrabi and turnip, but most seedlings were killed after they emerged. The spots on seedling stems were dark brown to black with badly constricted lesions at the soil line. The lesions were covered more densely with whitish to greenish-gray mycelium (Fig. 39). Spores were observed on the dead plants.

Seeds of the crucifers planted in soils that were mixed with pure cultures of the three Alternaria spp. although germinating fairly well, produced very few healthy plants and most of these were in soils containing the fungus A. brassicae. Pre-emergence damping-off accounted for a considerable loss of plants but post-emergence invasion of the seedlings caused the greatest reduction. Almost the reverse was true in considering the severity of the other Alternaria spp. in this respect. A. brassicicola caused about as much damping-off before the seedlings appeared above the soil surface as it did after they had emerged. The diseases produced under these circumstances were so severe as to practically eliminate the chance of obtaining any healthy plants. Alternaria raphani was also highly pathogenic resulting in practically no healthy seedlings. A larger per cent of plants was lost from post-emergence disease affecting the stems and cotyledons than was lost because of pre-emergence damping-off.

These fungi can be assigned the role of strong pathogenic organisms under highly favorable conditions for their development.

B. Seedling Inoculation

Twenty-eight day-old seedlings of each of the cruciferous varieties were atomized with a spore suspension of the pathogens, and the duplicate

sets of seedlings were atomized with water as a check. Few drops of Tween 80 was used as a wetting agent. All seedlings were placed in a moist chamber in a greenhouse for 12 hours. All were then left in the open. After five days the number of leaves infected were counted without any attempt to differentiate the numbers of lesions on them. All leaves inoculated with A. brassicicola and A. raphani showed many spots. Most infected leaves became blighted and were shed. The leaves inoculated with A. brassicae showed few spots and very few leaves were shed.

The results of inoculations on plants at this stage of development as shown in Table 22 indicate that all varieties are highly susceptible to be attacked by all three pathogens (Fig. 41). The inspection of the thirteen varieties of plants inoculated with A. brassicicola ranged from 96 to 100 per cent, whereas the percentage of infection caused by A. brassicae and A. raphani ranged from 81 to 96 and from 76 to 99 per cent, respectively. These results show that these pathogens cause severe damage to cruciferous seedlings of which A. brassicicola was the most pathogenic.

C. Inoculation of two-month old plants.

Spore suspensions of each of the three species of Alternaria spp. tested were added with a few drops of Tween 80, and were atomized onto the leaves of two-month old cruciferous plants on March 28, 1959. Greenhouse temperatures fluctuated between 15° and 28°C. The inoculated and check plants were placed separately on a soil-filled bench. Moisture was applied every four hours during the day by atomizers. The percentage of leaf infection, as shown in Table 23, was determined ten days after inoculation.

Of the thirteen varieties tested all except collards, kohlrabi, radish and turnip were highly susceptible to infection by A. brassicicola.

TABLE 22

PERCENTAGE OF INFECTION OF 28-DAY OLD SEEDLINGS INOCULATED WITH ALTERNARIA SPP.

Host	A. brassicae		A. brassicicola		A. raphani	
	No. Inoculated	Infection	No. Inoculated	Infection	No. Inoculated	Infection
Broccoli	47	94	57	100	81	96
Brussels spr.	28	89	58	100	51	78
Cabbage	70	93	82	100	59	76
Chinese cab.	44	91	70	100	28	93
Collards	80	90	89	100	76	88
Cauliflower	37	84	48	100	33	76
Kale	63	86	64	100	41	85
Kohlrabi	78	96	116	100	117	81
Mustard	58	86	96	100	107	89
Radish	170	81	153	100	170	99
Rape	153	91	130	99	97	85
Rutabaga	166	86	188	99	120	90
Turnip	94	82	138	96	173	89

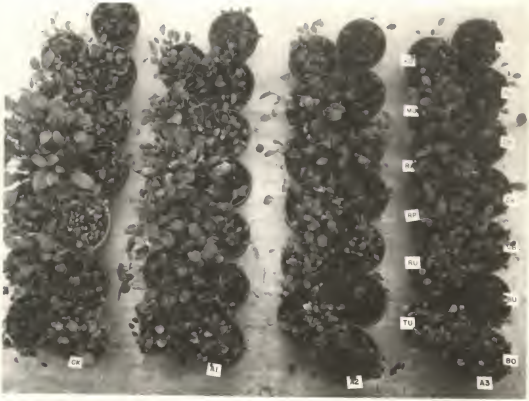

Fig. 41.--Twenty-eight day old seedlings of thirteen varieties five days after inoculation with Alternaria brassicae (A1), A. brassicicola (A2) and A. raphani (A3) compared with check on the left. The capital letters indicate cruciferous varieties as mentioned in Table 2.

TABLE 23

PERCENTAGE OF INFECTION OF TWO-MONTH OLD PLANTS INOCULATED WITH ALTERNARIA SPP.

Host	A. brassicae		A. brassicicola		A. raphani	
	No. Inoculated	Infection	No. Inoculated	Infection	No. Inoculated	Infection
Broccoli	29	93	13	100	40	88
Brussels spr.	21	52	31	100	35	29
Cabbage	22	94	66	100	23	69
Chinese cab.	9	89	17	94	8	52
Collards	33	73	21	72	35	60
Cauliflower	53	94	18	100	8	63
Kale	33	82	44	95	15	34
Kohlrabi	47	85	68	79	42	38
Mustard	7	100	3	100	10	100
Radish	22	73	24	83	31	94
Rape	36	93	29	97	19	74
Rutabaga	30	90	47	89	23	96
Turnip	4	100	5	60	7	43

The disease produced was severe except on turnip, which was only moderately affected. Of the plants inoculated with A. brassicae all showed highly susceptible except kohlrabi, kale, radish and collards which showed only moderate susceptibility, while brussels sprouts was the least susceptible. Mustard and radish plants infected by A. raphani produced large spots and in this respect were different from those infected by the other two pathogens. The spots on the other eleven hosts produced by this fungus remained less than 3 mm in diameter. The percentage of infection was much less by this fungus than those inoculated by the other two Alternaria spp. The lesions appeared as circular corky spots frequently surrounded by a yellow halo.

D. The susceptibility of cruciferous hosts in the field

The thirteen varieties of crucifers were planted in the field in three replications about fifteen to twenty feet apart. Five plants of each variety were grown in consecutive rows in each replication. The plants in the three replications from two to four months old were inoculated on October 6, 1959 with the three Alternaria spp. respectively. Observations were made on December 30, 1959, 86 days after inoculation. The diseases produced were identified in order to check natural infection as to the causal pathogens by microscopic identification of the spores produced on the lesions. Moderate to severe infection was found on plants infected by A. brassicicola. Likewise A. brassicae produced moderate infection and on many hosts was severe, but generally not as important as the preceding fungus. Infection by A. raphani was barely noticeable except on radish, where it was severely attacked and on mustard and broccoli where it was more than a trace. On several varieties no infection could be found..

The comparative amount of susceptibility of all cruciferous varieties tested to the pathogens is given in Table 24.

Cross Inoculation Test

Six selected isolates of A. brassicicola from broccoli, cabbage, Chinese cabbage, collard, mustard and turnip were recultured on potato-dextrose agar in Petri dishes for about two weeks. The cultures were flooded with distilled water and the spores were brushed off with a camel-hair brush. The spores were separated from the mycelium by sieving through a 50 mash-screen. Tween 80 as a wetting agent was added to the spore suspension. Five isolates of A. brassicae from broccoli, Chinese cabbage, collard, mustard, and turnip were recultured and the spore suspensions were prepared by the same manner as mentioned above. Only two suspensions, one from A. brassicicola isolated from collard and another from A. brassicae isolated from turnip were used as inoculum. These suspensions were atomized onto all the varieties of four-month old cruciferous crops grown in the field. Other suspensions of both pathogens were atomized on the leaves of two young Chinese cabbage plants which were about six-weeks old grown in the same pot in the greenhouse. Those suspensions were also atomized onto the varieties of young plants from which they were isolated. Two pots of Chinese cabbage plants were used as checks. All pots were kept in a plastic moist chamber for 48 hours.

All isolates of A. brassicicola isolated from different cruciferous hosts caused infection on Chinese cabbage plants in the greenhouse with somewhat similar virulence. Several spots appeared first as chlorotic or yellow in color and became water soaked, scalded-like, enlarged and rotted rapidly,

TABLE 24

SHOWING COMPARATIVE AMOUNTS OF DISEASES PRODUCED ON CRUCIFERS THROUGH INOCULATIONS WITH ALTERNARIA SPP.

Hosts	Amount of Disease Produced		
	A. brassicae	A. brassicicola	A. raphani
Broccoli	Moderate	Severe breaking of petiole.	Moderate
Brussels spr.	Moderate	Moderate	None
Cabbage	Severe on old leaves	Severe on head	None
Chinese cab.	Severe pod, leaves, stem	Severe on pod, leaves, stem	Trace
Collards	Severe on old leaves	Moderate few large lesions	None
Cauliflower	Severe	Severe	Trace
Kale	Moderate	Moderate	None
Kohlrabi	Severe, stem	Moderate	Trace
Mustard	Severe on pod, leaves, stem	Severe on pod, leaves, stem	Moderate
Radish	Severe on leaves, petioles, exposed root	Moderate	Severe petioles, exposed root
Rape	Moderate	Moderate	Trace
Rutabaga	Severe	Severe	Trace
Turnip	Severe petioles	Moderate	Trace

causing the whole leaf to collapse, dry out and drop. The pathogen isolated from collard was also pathogenic on all varieties of crucifers inoculated in the field. The infection was observed within two to four days as chlorotic or purplish spots that enlarged rapidly. There were some variations of number and size of spots on different hosts. Severe infections were observed on all varieties except radish and turnip, which were less susceptible.

The infection of Chinese cabbage plants which were inoculated in the greenhouse by A. brassicae was observed within four days as chlorotic areas which became yellowish-brown to gray. The lesions on leaves varied in number from none to five, with differences in size and shape on the plants inoculated with the same isolates. Infection was also found on all varieties of crucifers in the field inoculated with the turnip isolate. Severe infection was observed on Chinese cabbage, kohlrabi, mustard, radish, and turnip, less severe on broccoli, collard, cauliflower, rutabaga, and moderate on brussels sprout, cabbage, and kale.

The results of this experiment shows that isolates of the same fungus from the different hosts are similar and cause indistinguishable diseases on the same hosts. When the same isolate is used to inoculate the thirteen varieties of crucifers, a wide range of pathogenicity or host resistance was evident.

Chromatography

Amino acids are the elementary compounds involved in the nitrogen metabolism of fungi and other organisms. Cochrane (14) has discussed the results of studies on forms of amino acids used as carbon and nitrogen

sources for fungi. Amino acids may be required in media for optimum growth of some fungi. An amino acid may, however, allow good growth of one fungus but support only a slight growth of another. Paper chromatography has been utilized by several investigators to study the amino acids in plants. Waris (80) reported that the neomorph pattern of growth of Oenanthe aquatica (L.) Lam. was induced by critical concentration of amino acids, glycine and sustained by various other amino acids, by two pyrimidine derivatives and by an amine. He concluded that the amino acids are closely concerned with differentiation. Auclair and Maltais (4) stated that the resistance of Champion of England and the susceptibility of Perfection bean varieties to aphid infestation are due to the free amino acids contents. The differences of amino acids on healthy and diseased leaves of Acalypha indica Linn. were reported by Loloraya et al. (43). Woltz and Jackson (88) referred to the investigation of the United States Department of Agriculture workers to the effect that frenching of tobacco can be induced by furnishing plants with isoleucine and leucine. By one dimensional paper chromatography of healthy and yellow strapleaf diseased plants of chrysanthemum they found that the amount of amino acids in the diseased leaves was higher than that in healthy leaves. They believed that susceptibility might have been attributed to the excess of isoleucine and some other amino acids in susceptible chrysanthemum leaves.

The experiments were conducted in order to study the correlation of the amino acid contents of A. brassicae, A. brassicicola, and A. raphani and the thirteen varieties of cruciferous plants to the disease susceptibility.

The Pathogen Extracts:

Czapek liquid medium was prepared for fungous cultures. It was composed of the following materials:

Sodium nitrate	2.00 g
Potassium dibasic phosphate	1.00 g
Potassium chloride	0.50 g
Magnesium sulphate	0.50 g
Ferrous sulphate	0.01 g
Dextrose	30.00 g
Deionized water	1000.00 ml

The preparation of this medium followed Riker and Riker (61). A number of five-hundred-millliliter flasks were cleaned and rinsed with deionized water. Each flask contained 150 ml of medium. Four flasks were planted with a 3 mm disc of each pathogen grown in potato-dextrose agar while one was planted with a similar sterile agar disc as a check. The mycelial mats were removed after six weeks. The mats of A. brassicae were smaller and thinner than those of A. brassicicola and A. raphani. The cultures of each pathogen were filtered and dried at 44°C for two days. One gram of the dry weight of each pathogen was used for extraction as mentioned by Thompson et al. (72). The dry mat was boiled in 100 ml of 70 per cent ethanol for 10 minutes. The sample was triturated in a Waring Blendor for five minutes and was filtered. The filtrate was evaporated on a hot water bath until about 15 ml was left. The method of desalting by ion exchange resin, as recommended by Thompson et al. (73) was followed. Deionized water was prepared by passing distilled water through a deemizer containing Deeminite L-10. About 50 g of Dowex 50W-X2(200 to 400 mesh) was soaked in deionized water overnight and stirred with an equal volume of deionized water in a flask. The supernatant was decanted after thirty minutes. The process was repeated twice. The hydrogen form of Dowex

50W-X2 was heated in an oven for sixteen hours at 100°C with two volumes of 1/N sodium hydroxide. It was poured into a glass column, about 20x1 cm, with a short capillary absorbing end about 0.2 cm in diameter which was plugged with a small piece of glass wool. The resin in a column was about 7 cm deep. The resin was drained and washed with deionized water to remove the excess sodium hydroxide, then it was treated with five column volumes of 6/N hydrochloric acid. The hydrochloric acid was removed with deionized water until the effluent was free from chloride ion as tested by 1/N silver nitrate solution. The ammonium-form column was prepared from the hydrogen-form column by treatment with ten volumes of 2/N ammonium hydroxide. The resin column was then washed with boiled deionized water for about fifteen times, until the effluent reached a pH of 8 to 9. The sample was cooled to about 5° to 10°C on an ice bath, and then was poured on the column of ammonium resin. The effluent from this column was passed through the acid column later. Each column was rinsed with four successive 10-ml rinses of cold deionized water. Each resin column was eluted with 50 ml of 2/N ammonium hydroxide. Air pressure over the glass column was used during the process to speed up flowing. The eluates were combined and evaporated on a hot water bath until about 1 ml remained. The extracts were stored in the refrigerator at 0°C until used.

The plant extracts:

Healthy leaves of each variety of cruciferous plants grown in the field were collected. After the midveins had been removed they were cut into small pieces. Fifteen grams of each variety was extracted by boiling with 100 ml of 70 per cent ethanol for ten minutes. Leaves were triturated for five minutes and extracts were obtained by filtration. The filtrate

was evaporated down to about 15 ml on a hot water bath. The sample was stirred with 5 g of Dowex 50W-X2 for 5 minutes. Free amino acid content in the supernatant was checked by dropping the solution on a filter paper, which was dried and later developed with 0.2 per cent ninhydrin. A small amount of Dowex 50W-X2 was added to the sample and stirred again until a negative ninhydrin test was obtained. The supernatant was decanted. The remaining resin was eluted with 50 ml of 1/N ammonium hydroxide, stirred for a few minutes, and then filtered. The resin was then washed with 25 ml of 2/N ammonium hydroxide. Both filtrates were combined and evaporated down to about 1 ml on a hot water bath, and stored as those of the pathogen extracts.

Development procedure.

One-dimensional chromatograms of the pathogen and plant extracts were made. An 18 x 22 inch No. 1 Whatman filter paper was spotted with a platinum wire loop. About 4 ul of each pathogen extract was placed in each of a series of spots in a line, 1/2 inch below the 2 inch folding edge of the paper (about 1 ul at a time and allowing the spot to dry before repeating). The paper was equilibrated for 5 hours in the vapor of n-butanol: acetic acid:water (v/v 120:30:50) in a chromatocab before running the chromatogram with the same solvent. Eighty milliliters of solvent were used in each glass trough, and it was allowed to move in a descending fashion. The solvent reached the lower edge after 17 to 20 hours. The chromatogram was dried in 85-90°C oven for 15 minutes and then dipped into 0.2 per cent ninhydrin solution in acetone. The paper was dried again at the above temperature for 5 minutes. Chromatograms of plant extracts were prepared in the same manner except that 2 ul was placed in each spot.

Twenty-one standard amino acids were kindly supplied by Dr. T. E. Freeman, Agricultural Experiment Station, Gainesville, Florida. The result of the Rf values with n-butanol:acetic acid:water as the solvent is shown in Table 25.

The chromatogram of the pathogen extracts (Fig. 42) indicates the total presence of 7 different compounds in all three pathogens at 0.10, 0.19, 0.25, 0.32, 0.50, 0.56 and 0.66 Rf values. Each pathogen has two similar purple compounds at 0.19 and 0.25 Rf values. These spots were larger and darker purple in A. brassicicola extract than any others, indicating a greater amount of the compound in that pathogen. A. brassicae extract was also similar to A. brassicicola extract in that both of them contained the 0.32 and 0.56 purple compounds which were not present in the A. raphani extract. The extracts of the first two species differed by the presence of the 0.10 brown compound in A. brassicae extract and the 0.50 purple compound in A. brassicicola extract. The A. raphani extract did not contain these last two spots, but it showed a different purple one at the 0.66 Rf value. By the presence of spots as described above A. brassicae and A. brassicicola extracts are more similar to each other than either is to A. raphani.

Regarding the two purple spots that appeared in the check, the lower spot differed from the other spots of the pathogen extracts because of its 0.21 Rf value, which indicates that it is a different compound. The 0.32 compound which was present in the check extract was not present in the A. raphani extract. This indicates that the Czapek liquid medium in the check was contaminated, but not by the compounds used in the medium preparation.

TABLE 25

THE VALUES OF TWENTY-ONE STANDARD AMINO ACIDS WITH N-BUTANOL: ACETIC ACID:WATER SOLVENT AND NINHYDRIN DEVELOPING REAGENT

Compound	Rf	Compound	Rf
L-Leucine	0.66	L-Aspartic acid	0.24
L-Isoleucine	0.64	L-Serine	0.23
L-Phenylalanine	0.59	Glutamine	0.20
L-Tryptophan	0.55	L-Arginine monohydro-chloride	0.16
L-Valine	0.52	L-Histidine monohydro-chloride	0.15
L-Methionine	0.51	L-Asparagine	0.15
L-Tyrosine	0.43	L-Lysine	0.15
L-Proline	0.39	L-Cystine	0.09
L-Alanine	0.34	L-Cystine hydrochloride	0.06
L-Glutamic acid	0.29		
Thrionine	0.27		
Glycine	0.25		

A combination of 21 standard amino acids was spotted on both sides of the chromatogram of plant extracts, giving a roughly qualitative comparison of the amino acids in plant extracts with those in standard solutions.

Fig. 42.--Three replications of one-dimensional chromatogram with n-butanol:acetic acid:water of Alternaria brassicae (A1); A. brassicicola (A2); A. raphani (A3); and Czapek liquid medium (CK) extracts showing their amino acids.

According to the field observation as shown in Table 24, it can be assumed that the degrees of susceptibility of those cruciferous hosts to A brassicae, A. brassicicola and A. raphani may be divided into four categories as in Table 26. The interrelationship among amino acid contents of parasites and hosts and host susceptibility may be summarized as follows:

1. A. brassicae is more similar to A. brassicicola than to A. raphani. This fact seems to be correlated with the varietal susceptibility of hosts as shown on Table 26 in that the first two pathogens cause severe to moderate symptoms, whereas A. raphani caused severe damage only on radish, and susceptibility of other hosts as rated as trace or none. (See Table 26)
2. The 0.66 compound contained in the A. raphani extract (Fig. 42) corresponded to the distinct darkest spot at the same Rf value in the radish extract (Fig. 43). This correspondence is interesting in that this pathogen also causes severe damage only to radish.
3. Each plant extract contained several amino acids (Fig. 43). The spots lower from about Rf 0.3 on each column were larger and darker than the upper ones. There are some differences in color and size of spots; however, no specific correlation between occurrence of such spots and host susceptibility was detected.

TABLE 26

DEGREES OF SUSCEPTIBILITY OF 13 CRUCIFEROUS VARIETIES* IN THE FIELD INFECTED BY A. BRASSICAE, A. BRASSICICOLA AND A. RAPHANI

Susceptibility	A. brassicae	A. brassicicola	A. raphani
Severe	--,--,CB CH,CO,CU KO,MU,RA RU,TU	BO,BU,CB CH,--,CU --,MU,-- --,RU	RA
Moderate	BO,BU,-- KA,--,-- RP,--	--,--,CO KA,KO,RA RP,TU	BO,MU
Trace	-	-	CH,KA,KO RP,RU,TU
None	-	-	BU,CB,CH CO

*Thirteen varieties of crucifers are indicated as capital letters which were mentioned in Table 2.

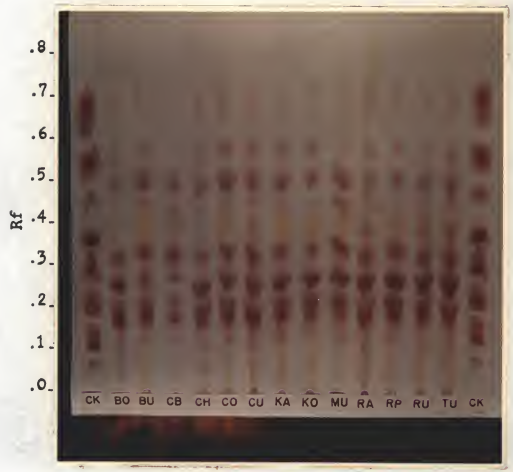

Fig. 43.--One-dimensional chromatogram with n-butanol: acetic acid:water of the 13 varieties of cruciferous extracts. Capital letters indicate host varieties as mentioned in Table 2, standard amino acids (CK).

SEASONAL DEVELOPMENT OF THE DISEASE

The pathogens are seed-borne and infected seeds are the most important source of primary infection in new lands. The pathogens are carried on the surface of seed as spores which remain viable for at least twenty months (60). Seed treated with calcium hypochlorite and corrosive sublimate reported by Chupp (13) have not been effective. He concluded that the mycelium of A. brassicae hibernated under the seed coat, and he recommended a hot water treatment. Rangel (60), by sectioning of infected seed, affirmed that both A. brassicicola and A brassicae were carried as latent mycelium under the seed coat. Primary infection also occurred from the fungus that had remained viable in plant debris in the fields. Atkinson (3) showed that A. raphani did not establish an overwintering inoculum in plant debris in the soil.

In Florida, the main crucifer-growing period begins in August when the temperatures, relative humidity and precipitation are the highest of the year. The earliest plant beds and fields are seeded in the fall. The growing season continues through the winter and spring, tapering off in May. This growing season is cool, less humid and dry. The crop plants are favored by these conditions, while the Alternaria spp. are not. Most of the cabbage crop is grown and harvested during the five-month period from November 1 to March 30. Collards, mustard and turnips are the principle crucifers grown in Florida in home gardens and in fields to supply local markets during the remaining months of the year. On these summer grown plants, A. brassicicola is more destructive and more frequently found

than A. brassicae which usually causes severe damage mostly on Chinese cabbage, mustard, radish, and turnips. Data accumulated from several experimental plantings of thirteen commercial varieties of crucifers which have been recorded over a period of two years showed that black leaf-spot occurred during every month of the year, and plants may become infected at any time during their development. Conidia are borne abundantly and disseminated readily by air current. Severe outbreaks have been found during the warmer months. Gray leaf-spot was less severe and usually occurred during the cooler part of the year.

Both pathogens are usually found infecting the same plant. If infection took place when the plants were young and the weather was favorable they were killed or suffered considerable damage. Heavily infected leaves are killed and fall off as exemplified with broccoli, radish, and turnips. If the weather becomes warm and dry, foliage leaves of Chinese cabbage and cabbage are shed, leaving bare heads that are often destroyed by these fungi. Chupp (13) observed that under warm and moist weather, turnip plants infected by gray leaf-spot disease died. Turnip plants growing in cool weather were less infected. Eddins (19) stated that frequent rains and heavy dews which kept plants wet until midmorning or longer caused rapid development and spread of the disease.

No natural infection caused by A. raphani has been reported in Florida. Infection in the field by inoculation took place on the crucifers after October 6, 1959. It was not commonly found after several months because the inoculated foliage was shed and secondary infection did not take place as commonly as the other Alternaria spp. infecting crucifers.

The climatological data made at the Agronomy Farm Weather Station, Gainesville, Florida, located about a mile away from the experimental field are given in graphic form (Fig. 44), and show the weather for the past two years as constituted by the combination of maximum and minimum temperatures, precipitation, relative humidity, and rainfall to numbers of rainy days per month for this period. The diseases of crucifers caused by Alternaria spp. are most serious in fall and spring than in summer because host plants are not abundant or in winter because the cool temperatures and dryness are unfavorable for the development of these fungi.

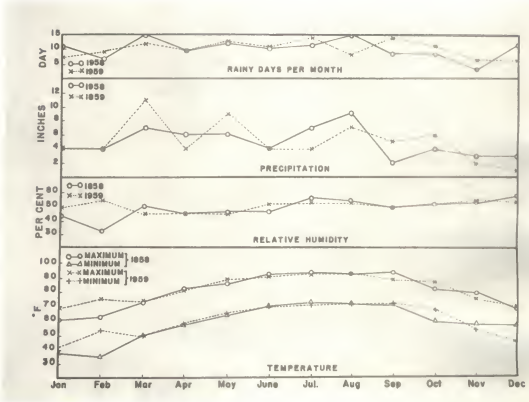

Fig. 44.--Temperatures, relative humidity, precipitation and number of rainy days per month in 1958-1959.

PATHOLOGICAL ANATOMY

Host Penetration

Host penetration of hyphae from germinated spores of all three pathogens was studied in the laboratory. Absorbent cotton swabs soaked in separate spore suspensions of each of the three pathogens were applied individually to the abaxial surface of different two-month old cabbage and broccoli leaves. The inoculated areas were marked with India ink. All plants were incubated in a moist chamber at 20°C controlled temperature. The inoculated leaves were sampled every six hours after a twelve-hour incubation period. The epidermis of the samples collected was removed by scalping with a razor blade and floated in water on glass slides. Frequently 70 per cent ethanol was used to remove some attached chloroplasts. These slides were warmed for a few seconds and stained with a 0.1 per cent solution of cotton blue dye in lactophenol and a cover slip was then placed over them for microscopical examination.

The penetration of hyphae of germinated spores of A. brassicicola and A. raphani took place directly through the epidermis of the samples collected 36 hours after inoculation. (Fig. 45, 46, 47). However, by the evidence of the lesions, A. brassicicola germinated and entered the host more rapidly than A. raphani and the infected areas were slightly larger and some host cells were already affected by the hyphae which penetrated through the epidermis. Only once was a hypha observed penetrating through an open stoma (Fig. 45). Usually one to three hyphae emanated from a

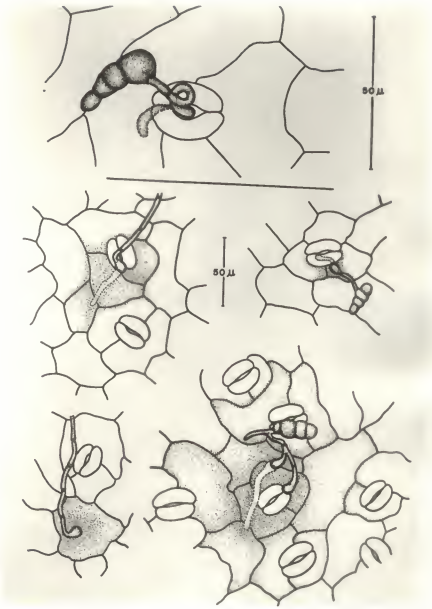

Fig. 45.--Camera lucida drawing at the top shows the penetration of the hyphae of Alternaria brassicicola through a stomata and through the epidermal cells of a cabbage leaf after 36 hours in 20°C. The shaded areas indicate the affected host cells.

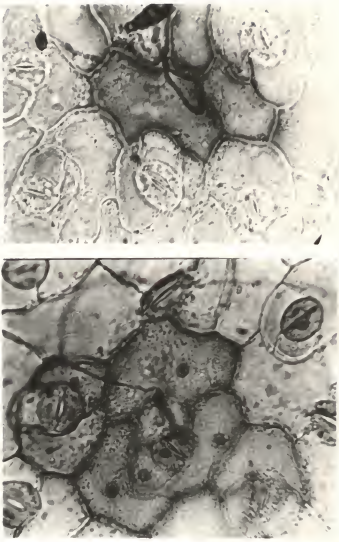

Fig. 46.--Direct penetration of Alternaria brassicicola through the epidermis of a cabbage leaf inoculation after 36 hours.

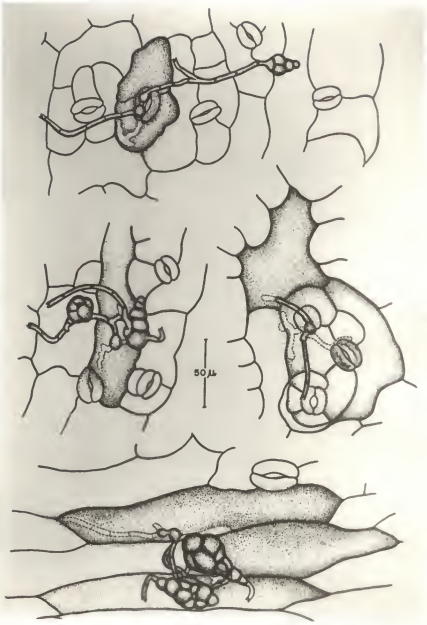

Fig. 47.--Camera lucida drawing shows direct penetration of the hyphae of Alternaria raphani through the epidermal cells of a broccoli leaf 36 hours after inoculation. The shaded areas indicate the affected host cells.

single spore. They branched frequently and grew over the surface of the leaves. The terminal portion of hypha enlarged and formed an appressorium prior to or during the process of penetrating. The size of the hyphae developed in the host from the port of entry were larger than the outside portion. One to several cells surrounding the penetrated cell soon became brown and are easily distinguished from the surrounding healthy cells.

The stomatal penetration of hyphae of A. brassicae was observed in a sample collected 60 hours after inoculation. The formation of an appressorium occurred prior to entrance through the stomata regardless of whether open or closed (Fig. 48). Three to five hyphae were regularly produced by a spore. The beak cells mechanically broke off from spores usually germinated and caused infection. After penetration took place, the hyphae swelled and formed rhizome-like structures in the stomatal chamber from which hyphae developed. Germ tubes were not attracted to the stomata, as they were found passing across them and then penetrating the host directly.

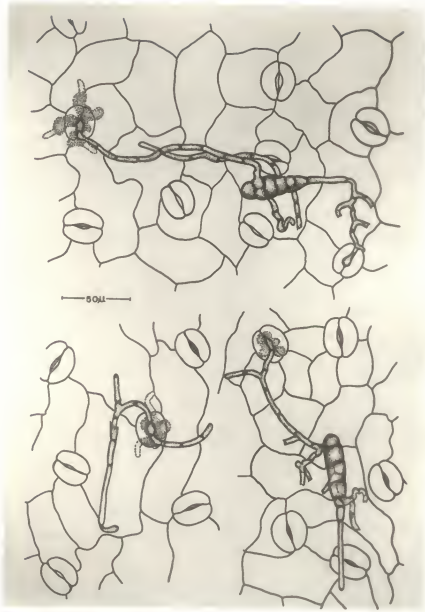

Fig. 48.--Camera lucida drawing shows the penetration of the hyphae of Alternaria brassicae through the stomata of a cabbage leaf after 60 hours in 20°C. At left the beak of a spore germinated and caused infection.

SUMMARY

1. Three Alternaria spp. pathogenic on crucifers cause black leaf-spot, gray leaf-spot and radish leaf-spot respectively.
2. Black leaf-spot and gray leaf-spot cause considerable losses of crops in the southern states, whereas radish leaf-spot had not been observed.
3. Symptoms of each disease on thirteen varieties of crucifers are described. Interaction of the pathogen and host-variety show variations. Spore identification is frequently required to verify the causal organism of the disease.
4. The valid names of these pathogens are Alternaria brassicicola (Schw.) Wilt., A. brassicae (Berk.) Sacc. and A. raphani Groves and Skolko.
5. The morphology of the pathogens in culture and on host tissues is described. There are distinct differences in body and beak measurements, average size, shape, number of transversal and longitudinal septa and catenulation.
6. Alternaria brassicicola grows and sporulates well on a wide range of artificial agar media. The growth and sporulation of A. brassicae is slightly poorer. Growth and sporulation of A. raphani was obtained best in most cruciferous leaf decoction agar media although few spores are produced. The density of the thallus of colonies and sporulation of all three pathogens

decreased as the amount of leaf decoction per liter in the medium decreased from 400 to 6.5 g.

7. The optimum temperature for growth in potato-dextrose agar medium of A. brassicicola is 24 to 28°C while those of A. brassicae and A. raphani are 20 to 24°C and 24 to 28°C respectively.

8. The optimum hydrogen-ion concentration for growth in potato-dextrose agar medium of A. brassicicola is 6.0 to 8.0 pH values while those of both A. brassicae and A. raphani are 7.1 to 8.0 pH values

9. Zonation formation on A. brassicicola in culture is produced by exposure to light and darkness. No zonation is formed in culture growing in continuous light or continuous darkness. Better sporulation is found in cultures exposed to continuous darkness than those exposed to continuous light.

10. Pre-emergence and post-emergence damping-off occurs from the contaminated soil. In the field leaf spotting is especially severe on seedlings becoming less important as the plants mature. Isolates of A. brassicicola and A. brassicae collected from different cruciferous hosts produced infection on thirteen varieties of crucifers tested.

11. There is a correlation of susceptibility of the cruciferous varieties and amino acids contained in each pathogen. Severe to moderate susceptibility of cruciferous varieties to A. brassicicola and A. brassicae and the similarity of amino acids contained in these two pathogens is apparent. The susceptibility

of hosts to A. raphani was severe only on radish. One amino acid contained in A. raphani extract was found corresponding to that of the radish extract.

12. Primary sources of infection are the infected seed and air-borne spores.

13. Direct penetrations of A. brassicicola and A. raphani occurred within 36 hours. Stomatal penetration observed by A. brassicae 60 hours after inoculation.

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BIOGRAPHICAL DATA

Winit Changsri was born in Bangkok, Thailand, on October 24, 1925. he was graduated from Matayomyothinburana High School, Bangkok, in 1941, and from the Pre-University of Kasetsart University, Chiengmai, in 1945. In 1950, he was granted a degree of Bachelor of Agriculture with honors by Kasetsart University.

He was employed in the section of Entomology, Plant Industry Division, Department of Agriculture for two years from 1950. He was called to serve as a soldier by the military air force for six months. After his discharge he came back to continue his work in the Plant Quarantine Section of the same Division.

He was married in 1953 and has two children. He received a Thai government scholarship to study plant pathology in the United States in 1955. He arrived at the New York airport on March 1, 1956 and entered the University of Florida in September of the same year as a special graduate student. He became a graduate student in the second semester, 1956-57. He was awarded the degree of Master of Science in February, 1958.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 6, 1960

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